European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Glyphosate

Volume 3 – B.6.5 (AS)

Rapporteur Member State : Assessment Group on Glyphosate (AGG) consisting of FR, HU, NL and SE

Version History

When	What
2021/06	Initial RAR

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

B.6.5.1. Long-term toxicity – rat, study 1

1. Information on the study

Data point:	CA 5.5/001
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat
Report No	2060-0012
Document No	NA
Guidelines followed in study	OECD 453 (1981), JMAFF Guideline 2-1-16 (2005), US OPTTS 870.4300 (1996)
Deviations from current test guideline (OECD 453, 2018)	The following deviations from the current OECD test guideline were noted: - Organ weight measurements did not include the thyroid/parathyroid - The histopathology did not include the cervix, coagulating gland and the lacrimal gland.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid, Category 2a **Conclusion AGG:** Some minor deviations were noted compared to the current OECD test guideline. However, these are not considered to be critical and therefore the study is considered to be acceptable.

Full summary

The chronic toxicity and carcinogenic potential of Glyphosate technical was assessed in a 24-month feeding study in 51 male and 51 female Wistar rats at dietary concentrations of 0, 1500, 5000 and 15000 ppm (equal to achieved

dose levels of 0, 85.5, 285.2, 1077.4 mg/kg bw/day in males and 0, 104.5, 348.6 and 1381.9 mg/kg bw/day in females) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/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.

Observations covered clinical signs, behavioural assessment, functional observations, body weight, food consumption, ophthalmology, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

The treatment-related findings of this study were elevations in plasma electrolyte values for both sexes at 18 months. Elevations in alkaline phosphatase activity were seen at 6, 12 and 18 months. Histopathological examinations revealed at 15000 ppm a significant difference in the site of mineral deposition within the kidneys compared with controls. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia; which is considered a consequence of decreased mineral deposition. An increase in severity of adipose infiltration into the bone marrow was observed. In high dose males, skin effects including areas of necrosis/giant cell reaction to keratin and keratoacanthoma were observed.

The NOAEL is concluded to be 5000 ppm (equal to 285.2 mg/kg bw/day in males and 348.6 mg/kg bw/day in females).

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7 % w/w
Stability of test compound:	No data

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar Han Crl:WI (GLx/BRL/Han) IGS BR
Source:	[redacted]
Age:	5 – 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 112 – 183 g, females: 98 – 150 g
Acclimation period:	At least ten days
Diet/Food:	Rat and Mouse SQC Ground Diet No.1 (BCM IPS Ltd., London, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-09-01 to 2007-08-31

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 51 Wistar rats per sex received daily dietary doses of 0, 1500, 5000 and 15000 ppm (equivalent to mean achieved dose levels of 0, 95.0, 316.9 and 1229.7 mg/kg bw/day) Glyphosate technical. To ensure that a received dose of 1000 mg/kg bw/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.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet for 19 minutes at a constant speed. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test substance in the diet was determined in an in-house stability study. The homogeneity and achieved concentrations of the test substance preparations was determined at monthly intervals until Week 26, and in 3-month intervals thereafter.

Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes once and for pre-terminal deaths twice a day. A routine clinical observation session including veterinary examination was made weekly, including palpation for new or existing masses. Ophthalmic examination was done at the start of the study in all satellite animals and at Week 50 in ten satellite animals per sex of the control and high dose group. Prior to treatment and at weekly intervals thereafter all satellite animals were observed for behavioural toxicity.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination as well at terminal kill.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently for one week in each four weeks until termination.

Water consumption
Water intake was observed daily, for each cage group, by visual inspection.

Haematology and clinical chemistry
Haematological examinations were performed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. The following parameters were measured: haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, prothrombin time, and activated partial thromboplastin time.

Blood chemical investigations were performed on ten animals per sex from the satellite groups at 6 and 12 months and from the main groups at 18 and 24 months. The following parameters were determined: urea, glucose, total protein, albumin, albumin/globulin ratio, sodium, potassium, chloride, calcium, inorganic phosphorus, ASAT, ALAT, alkaline phosphatase, creatinine, total cholesterol, total bilirubin, and cholinesterase.

Urinalysis
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. The following measurements were made: specific gravity, volume, pH, protein, glucose, ketones, blood, urobilinogen, reducing substances and microscopic examination of sediment.

Sacrifice and pathology
Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as well for all animals found dead or killed in extremis.

The following organ weights were determined from 10 rats per sex and main group and from all satellite animals: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testis and thymus.

Tissue samples were taken from the following organs: adrenals, aorta (thoracic), bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions including palpable masses, head (pharynx, nasopharynx, paranasal sinuses), heart, Harderian gland, ileum (incl. Peyer's patches), jejunum, kidneys, liver, lungs (with bronchi), lymph nodes (cervical and mesenteric), mammary gland, muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary), sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina. A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

Histopathological examination was initially carried 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.

Statistics
Where appropriate quantitative data was analysed by the ProvantisTM Tables and Statistics Module. For each variable, the most suitable transformation of the data was found; the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANCOVA and Bartletts's test. The transformed data was analysed to find the lowest treatment level that shows a significant effect, using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response is found, but the data shows non-homogeneity of means, the data will be analysed by a stepwise Dunnett (parametric) or Steel (non-parametric) test to determine significant differences from the control group. Finally, if required, pair-wise tests are performed using the Student t-test (parametric) or the Mann-Whitney U test (non-parametric).
Histopathology data were analysed using the following methods to determine significant differences between control and treatment groups for the individual sexes.

1. Chi squared analysis for differences in the incidence of lesions occurring with an overall frequency of 1 or greater.
2. Kruskal-Wallis one way non-parametric analysis of variance for the comparison of severity grades for the more frequently observed graded conditions.

II: RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Stability assessment demonstrated that the test material preparations in the diet were stable for at least six weeks.

Analyses for achieved concentrations showed that the diet preparations were within an acceptable range. On one occasion the achieved concentrations of the low, mid and high-dose group were 79 %, 83 %, and 87 %, respectively. At week 2 the concentration in the mid dose group was 112 %. However, these isolated deviations from the nominal range were still considered to be acceptable.

B. MORTALITY
No significant treatment-related effects on mortality were observed during the study.
The numbers of pre-terminal deaths in the main group are displayed in the table below:

Table B.6.5.1-1 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al.*, 2009): Cumulated mortalities after 104-week dietary exposure to Glyphosate technical

	Dose group (ppm)			
Sex	**0**	**1500**	**5000**	**15000-24000**
Male	12	14	13	6
Female	14	17	15	12

C. CLINICAL OBSERVATIONS

No significant treatment-related clinical observations occurred during the study.

There were no treatment-related effects on behavioural assessments, functional performance tests or sensory reactivity assessments observed.

D. BODY WEIGHT

Body weights were not statistically significantly changed in any dose group. At the end of the dosing period the body weight of males/females of the high dose group was reduced by 7%/3 % compared to the control without being statistically significant. Body weight gains were partly changed in dose groups compared to the control. However, the variation of body weight changes was generally very high and increases as well as decreases were observed comparing control and dose groups and the effect on overall body weight gain (week 1-104) was only slight (<10%). In conclusion, the changes in body weight and body weight gains was not considered an adverse effect.

Table B.6.5.1-2 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al.*, 2009): Intergroup comparison of body weights

	Dose group (ppm)							
	0		**1500**		**5000**		**15000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	141 ± 17	123 ± 11	142 ± 17	123 ± 10	141 ± 17	122 ± 10	141 ± 16	123 ± 10
Week 13	393 ± 40	228 ± 18	401 ± 42	233 ± 18	397 ± 41	229 ± 17	379 ± 34	227 ± 15
Week 52	544 ± 55	289 ± 38	554 ± 62	294 ± 33	554 ± 55	290 ± 35	511 ± 51	281 ± 26
Week 104	618 ± 83	362 ± 67	641 ± 102	375 ± 61	648 ± 81	378 ± 51	577 ± 66	354 ± 49

Table B.6.5.1-3 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al.*, 2009): Intergroup comparison of body weight gains

	Dose group (ppm)							
	0		**1500**		**5000**		**15000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight gain [g]								
Week 1-2	39.6 ± 5	19 ± 7	40.6 ± 4	21 ± 4	39.5 ± 8	20 ± 4	37.2 ± 4** (91)	19 ± 6
Week 12-13	7.8 ± 3.4	1.2 ± 3.9	10.7 ± 16.5* (137)	2.1 ± 4.1	8.5 ± 3.1* (109)	2.5 ± 3.8	9.2 ± 3.4* (118)	2.6 ± 4.5
Week 52-53	-1.32 ± 3.5	-2.2 ± 7.1	-0.48 ± 4.3	-0.29 ± 5.7	-0.20 ± 6.9	1.2 ± 7.0	-1.5 ± 5.0	-1.3 ± 6.5
Week 101-104	3.0 ± 11.6	-1.8 ± 12	-4.4 ± 28.7	1.4 ± 12	-1.1 ± 23.7	2.5 ± 9	1.22 ± 14.1	1.5 ± 7

** Statistically significant difference from the control group mean at the 1 % level (Student's t-test, two-sided)
* Statistically significant difference from the control group mean at the 5 % level (Student's t-test, two-sided)
() percent of control (only indicated when statistically significant)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption or food efficiency for either sex noted during the study.

The group mean achieved doses are summarised below.

Table B.6.5.1-4 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al.*, 2009): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)		Mean achieved dose level (mg/kg bw/day)	
			Males	Females
1 (control)	0			
2 (low)	1500		85.5	104.5
3 (mid)	5000		285.2	348.6
4 (high)	15000	Week 1-11	1077.4	1381.9
	17000	Week 12-15		
	19000	Week 16-26		
	21000	Week 27-39		
	24000	Week 40-104		

The results show a higher test material intake for females when compared to males for each dose level. The mean intake values represent the combination of satellite and main group values.

F. WATER CONSUMPTION

There were no treatment-related effects on water consumption during the study.

G. OPHTHALMOSCOPY

There were no treatment-related effects observed.

H. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

At 3 months no statistically significant effect on haematological parameters were observed.

At 6 months a significant increase in haematocrit was observed in the low dose males but not in the mid or high dose and therefore considered incidental. MCHC was slightly reduced in mid and high dose males, but no clear dose response relationship was observed (35.05, 34.91, 34,85, 34,83 at 0, 1500, 5000 and 15000/24000 ppm). In addition, activated partial thromboplastin time was increased in males but again without a dose response relationship (14.05, 15.67, 15.07, 15.97 at 0, 1500, 5000 and 15000/24000 ppm). In females the only significant effect observed was a slight increase in platelet count (677.4 versus 602.4 in controls).

In 12 month males no statistically significant effect was observed. In females in the mid and high dose, a very slight but statistical decrease in haemoglobin was observed. However, the effect lacked a dose-response relationship (14.578, 14,345, 14,278 and 14,238 at 0, 1500, 5000 and 15000/24000 ppm).

At 18 months the only significant effect observed in males was an increase in neutrophils in the mid and high dose. In females a decrease in white blood cell count (3.12, 2.48, 2.71, 2.48 at 0, 1500, 5000 and 15000/24000 ppm) and a decrease in activated partial thromboplastin time was observed (14.19, 13.06, 12.63, 13.40 at 0, 1500, 5000 and 15000/24000 ppm). However, both effects lacked a dose-response relationship.

At 24 month the only significant a increase in monocytes in low dose males. No effect was observed in mid or high dose males or females.

All variations were considered to be incidental and unrelated to treatment because of the lack of either a true dose response, a consistent change throughout the study, a lack of progression of change with time and/or lack of concomitant effect in both sexes.

Clinical chemistry
At the highest dose level there was an increase in alkaline phosphatase activity for satellite group males and females compared with controls at 6 and 12 months. Main group males were also affected at 18 months. A slight increase in alkaline phosphatase activity was seen for satellite group males at 12 months. Values for all alkaline phosphatase activity values are presented as follows:

Table B.6.5.1-5 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al.*, 2009): Alkaline phosphatase activity (IU/L)

	Dose level							
	Control		**Low**		**Intermediate**		**High**	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Month 6 (Satellite)	87.8	49.6	94.5	62.9	103.4	62.0	**128.5** (+46%)	**91.9** (+85%)
Month 12 (Satellite)	87.7	46.1	96.5	59.7	**116.3***	58.1	**140.2** (+59%)	**91.3** (+98%)
Month 18 (Main)	93.3	65.7	110.5	55.8	110.9	70.9	**125.0*** (+34%)	92.7 (+41%)
Month 24 (Main)	107.2	66.0	98.8	58.5	101.0	81.7	111.9 (+4%)	86.8 (+32%)

* $p < 0.05$; ** $p < 0.01$

The lack of a consistent effect for females and the absence of any histopathological correlation does suggest this to be of limited toxicological importance. However, it is noted that in the high dose group the change is above the limit of 50% at the 6 and 12 month time point which is used by JMPR as a starting point to consider adversity.

At the 18 month evaluation there was an increase in plasma electrolytes for both sexes.
Sodium and chloride values for males and females and calcium values for males only were increased compared with controls. Female calcium levels were lower than controls. These elevations/decrements were also observed at lower dose levels but there was no clear trend over time. In addition at the 12 month evaluation for satellite females a lower sodium value was seen for females. Values for all sodium, calcium and chloride values are presented as follows:

Table B.6.5.1-6 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat [redacted] *et al.*, 2009): Calcium, sodium and chloride values (mmol/L)

	Dose level							
	Control		**Low**		**Intermediate**		**High**	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Calcium								
Month 6 (Satellite)	2.587	3.693	2.701	3.752	2.617	3.637	2.508	**3.604*** (-2%)
Month 12 (Satellite)	2.530	2.602	2.543	2.587	2.458	2.475	2.514	2.483
Month 18 (Main)	2.231	2.775	2.523	**2.645*** (-5%)	2.656	**2.554**** (-8%)	2.598	**2.468**** (-11%)
Month 24 (Main)	2.431	2.293	2.487	2.396	2.511	2.288	2.297	2.347
Sodium								
Month 6 (Satellite)	152.8	153.7	153.8	153.1	151.8	152.7	154.0	152.1
Month 12 (Satellite)	149.5	149.0	150.4	148.9	149.1	148.6	15.2	**147.2* (-1%)**
Month 18 (Main)	145.6	144.7	**148.0** (+2%)**	**148.8** (+3%)**	**150.7** (+4%)**	**150.0** (+4%)**	**150.9** (+4%)**	**149.4** (+3%)**
Month 24 (Main)	149.1	146.1	147.9	147.2	149.1	146.6	150.1	146.7
Chloride								
Month 6 (Satellite)	107.7	105.8	107.1	106.1	107.0	106.1	108.5	106.7
Month 12 (Satellite)	105.6	103.9	105.1	104.8	104.3	104.7	105.9	104.2

Table B.6.5.1-6 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al*., 2009): Calcium, sodium and chloride values (mmol/L)

	Dose level							
	Control		**Low**		**Intermediate**		**High**	
Timepoint	♂	♀	♂	♀	♂	♀	♂	♀
Month 18 (Main)	103.3	101.8	**105.8** (+2%)**	**104.2** (+2%)**	**105.8** (+2%)**	**106.4** (+4%)**	**107.6** (+4%)**	**107.8** (+6%)**
Month 24 (Main)	104.5	103.4	104.4	103.1	104.3	102.2	105.4	102.8

* $p < 0.05$; ** $p < 0.01$

At the intermediate and low dose level similar findings to the highest dose level were seen for plasma electrolytes at the 18 month evaluation, but not at the 24 month evaluation. Whilst these observations were seen at the highest dose level, the lack of effect at the 24-month observation point, the slight nature of the changes and the effect being limited to one sex (for calcium only) does make the toxicological significance questionable.

All other differences were isolated in their finding and are therefore not toxicologically relevant.

I. URINALYSIS

There were no treatment-related effects observed.

J. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights

No effects on organ weight values were observed.

Histopathology

Adipose infiltration of the bone marrow was seen for the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity (interim death: 11/12, 14/14, 10/13, 6/6 males at 0, 1500, 5000, 15000 ppm; 6/14, 16/17, 14/15, 8/12 females at 0, 1500, 5000, 15000 ppm; terminal sacrifice: 38/39, 35/37, 36/38, 45/45 males at 0, 1500, 5000, 15000 ppm; 36/37, 34/34, 36/36, 38/39 females at 0, 1500, 5000, 15000 ppm). However, greater effects were seen among male rats dosed at the highest level and this attained statistical significance for terminal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow cellularity 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 but among premature deaths for animals of both sexes at the intermediate level and only low-dosed females. 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 both sexes and was more prevalent among female rats (terminal sacrifice: 12/39, 10/37, 9/38, 5/44 in males at 0, 1500, 5000, 15000 ppm; 28/37, 27/34, 25/36, 3/39 in females at 0, 1500, 5000, 15000 ppm); however corticomedullary mineralisation was seen in female rats only (3/37, 0/34, 0/36, 19/39 in females at 0, 1500, 5000, 15000 ppm). Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary 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 on 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.

An apparent increase in skin keratoacanthoma was observed in high dose males at terminal sacrifice. In the interim group 1 case was noted in controls, but not in the other dose groups. The overall incidence was therefore 2 in controls and 6 in high dose males.

Table B.6.5.1-7 Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat ([redacted] *et al.*, 2009): histopathology

	Dose level							
	Control		**Low**		**Intermediate**		**High**	
	♂	♀	♂	♀	♂	♀	♂	♀
Adipose infiltration of bone marrow (interim death)								
Absent	1	8	0	1	3	1	0	4
Minimal	5	3	7	7	5	6	2	2
Slight	3	3	1	3	0	2	1	3
Moderate	1	0	4	3	2	5	2	2
Marked	2	0	2	3	3	1	1	1
Adipose infiltration of bone marrow (terminal sacrifice)								
Absent	1	1	2	0	2	0	0	1
Minimal	21	3	25	8	21	5	15	6
Slight	16	15	9	9	12	8	25	9
Moderate	1	18	1	17	2	23	5	23
Marked	0	0	0	0	1	0	0	0
Mineralisation renal pelvis (terminal sacrifice)								
Absent	27	9	27	7	29	11	39	36
Present	12	28	10	27	9	25	5	3
Corticomedullary mineralisation (terminal sacrifice)								
Absent		34		34		36		20
Minimal	0	3	0	0	0	0	0	19
Hyperplasia pelvic/papillary epithelium (terminal sacrifice)								
Absent	37	29	34	19	37	30	43	37
Minimal	2	4	3	13	0	5	2	2
Slight	0	4	0	2	1	1	0	0
Skin (interim and terminal sacrifice)								
Areas of necrosis/ giant cell reaction to keratin	1	0	2	0	1	0	7	0
keratoacanthoma	2	0	3	0	0	0	6	0

Neoplastic changes
No significant effects associated with tumour development were observed.

Assessment and conclusion by applicant:
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 24000 ppm (corresponding to 1229.7 mg/kg bw/day for combined sexes). It is concluded that glyphosate technical is not carcinogenic in rats.

Assessment and conclusion by RMS:
Based on the observed increase in alkaline phosphatase, adipose infiltration of the bone marrow and kidney findings which of are of equivocal relevance in both sexes and the skin effects including areas of necrosis/giant cell reaction to keratin and keratoacanthoma observed in high dose males the NOAEL is concluded to be 5000 ppm (equal to 285.2 mg/kg bw/day in males and 348.6 mg/kg bw/day in females).
This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.5.2. Long-term toxicity – rat, study 2

Data point:	CA 5.5/002

Report author	[redacted]
Report year	2001
Report title	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats
Report No	[redacted]/PR1111
Document No	NA
Guidelines followed in study	OECD 453 (1981), EEC B.33 (1988), MITI (1992), US OPTTS 870.4300 (1998)
Deviations from current test guideline (OECD 453, 2018)	The following deviations from the current OECD test guideline were noted: - Organ weights of thyroid/parathyroid was not determined - Histopathological examination of coagulating glands and vagina were not performed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid, Category 2a **Conclusion AGG:** Some minor deviations were noted compared to the current OECD test guideline. However, these are not considered to be critical and therefore the study is considered to be acceptable.

Full summary

The chronic toxicity and carcinogenic potential of glyphosate acid was assessed in a 24-month feeding study in 52 male and 52 female Wistar rats with 0, 2000, 6000 and 20000 ppm (equal to dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females). In addition, three satellite groups with 12 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.

Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

Treatment related findings in this study were found in the liver and kidney and were confined to animals (predominantly males) fed 20000 ppm glyphosate acid. There were a number of changes in males and females fed 20000 ppm, notably renal papillary necrosis, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance. Despite the findings at 20000 ppm, survival was better in males fed 20000 ppm than in the controls and lower dose groups.

The NOAEL is 6000 ppm (equal to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females) based on the observed clinical chemistry changes and histopathological findings observed in the kidney, liver and prostate.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid (technical material)
Description:	White solid
Lot/Batch #:	P30
Purity:	97.6 % w/w
Stability of test compound:	At least 2 years when stored at -20 °C

2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Wistar (Alpk:APfSD)
Source:	[redacted]
Age:	3 weeks (on delivery)
Sex:	Males and females
Weight at dosing:	Males: 155.0 – 156.6 g (mean values); females: 136.0 – 138.4 g (mean values)
Acclimation period:	At least 10 days
Diet/Food:	CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1998-04-07 to 2000-05-07

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 52 Wistar-derived rats per sex received daily dietary doses of 0, 2000, 6000 and 20000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females).
A further twelve animals per sex were added to each group and were designated for interim kill after one year to study chronic toxicity and non-neoplastic histopathological changes.

Test diets were prepared in 60 kg batches by mixing a known amount of the test substance with 1 kg of basal diet. This pre-mix was then added to the remainder of the 60 kg batch of basal diet and mixed thoroughly. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Clinical observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study, at Week 52 and prior to termination. A functional observational battery including motor activity was conducted in Week 52 in animals allocated to the chronic toxicity assessment of the study.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 15 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 14, once in week 16 and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 13 animals per sex and group at Week 14, 27, 53, 79 and at termination. Different animals were used for the tail vein haematology and clinical chemistry samples.

The following blood parameters were measured: haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time. The following clinical chemistry parameters were measured: alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Week 13, 26, 52, 78 and prior to termination. The following parameters were determined: volume, abnormal colour and appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, and blood.

Sacrifice and pathology
Necropsy was conducted on all animals. The following organ weights were determined from all animals surviving to scheduled termination: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes and uterus.

Tissue samples were taken from the following organs: adrenals, aorta, bone and bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymides, eyes (retina, optic nerve), gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, lachrymal gland, larynx, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, muscle, oesophagus, ovary, pancreas, pharynx, pituitary, prostrate, rectum, salivary glands (submandibular, parotid), sciatic nerve, seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics
All data were evaluated using analysis of variance and/or analysis of covariance for each specified parameter using the MIXED procedure in SAS (1996). Kaplan-Meier survival estimates (Kaplan and Meier, 1958) were calculated separately for each sex and treatment group.
The overall incidence of each tumour type was considered by comparing each treated group and the control group using Fisher's Exact Test. In addition, a test for trend with group number was performed using the Cochran-Armitage Test described in Gart *et al*. (1986). Analyses were carried out for all animals, intercurrent deaths and at terminal kill.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
The mean achieved concentrations of glyphosate acid in each dietary preparation were within 10% of the nominal concentration and the overall mean concentrations were within 1% of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 2% of the overall mean for the 20000 ppm group and within 4-9% of the overall mean for the 2000 ppm group.

The stability tests determined at 2000 and 20000 ppm showed that the test substance stability was satisfactory at room temperature and when stored at -20 °C for at least 45 days which covered the period of use in the current study.

B. MORTALITY
The male groups were terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25 % (criteria for termination of the study). Statistically significantly better survival was observed in males fed 20000 ppm than in the other groups. A statistically significant overall trend was also observed for males.

The female groups survived to scheduled termination and there were no significant differences in mortality between the groups.

The survival rates are displayed in the table below.

Table B.6.5.2-1 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Survival rates during up to 104-week dietary exposure to glyphosate technical

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Week 13	0.98	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 26	0.95	1.00	1.00	1.00	0.98	0.98	1.00	1.00
Week 39	0.94	1.00	1.00	1.00	0.97	0.98	1.00	1.00
Week 52	0.91	1.00	0.97	1.00	0.97	0.98	0.98	0.98
Week 56	0.89	1.00	0.93	1.00	0.93	0.98	0.98	0.98
Week 60	0.87	1.00	0.92	1.00	0.91	0.97	0.98	0.97
Week 64	0.87	1.00	0.90	0.98	0.91	0.95	0.98	0.97
Week 68	0.87	0.94	0.88	0.96	0.87	0.95	0.98	0.95
Week 72	0.85	0.94	0.84	0.96	0.85	0.93	0.97	0.91
Week 76	0.81	0.94	0.80	0.92	0.82	0.89	0.97	0.91
Week 80	0.73	0.88	0.78	0.87	0.72	0.89	0.89	0.83
Week 84	0.69	0.85	0.67	0.83	0.63	0.89	0.85	0.83
Week 88	0.64	0.81	0.57	0.81	0.59	0.83	0.77	0.81
Week 92	0.56	0.79	0.50	0.81	0.53	0.81	0.71	0.80
Week 96	0.50	0.73	0.46	0.73	0.53	0.77	0.66	0.72
Week 100	0.40	0.69	0.44	0.63	0.42	0.77	0.56	0.66
Week 104	—*	0.62	—*	0.56	—*	0.77	—*	0.57

* Terminated in Week 100 because survival in the control, low and mid dose groups was approaching 25 % (criteria for termination of the study).

C. CLINICAL OBSERVATIONS

At 20000 ppm there was a treatment related increase in the incidence of red-brown staining of tray papers, particularly in males.

There were no other treatment-related clinical observations.

There were also no treatment-related effects noted in the functional observational battery.

D. BODY WEIGHT

The bodyweights of the animals fed 20000 ppm glyphosate acid were statistically significantly lower than controls throughout the study. The maximum reduction from control values was approximately 5% for males and 8% for females.

There were no treatment related effects in animals fed 2000 or 6000 ppm glyphosate acid.

Table B.6.5.2-2 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats [redacted], 2001): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	155.9 ± 14.8 (N = 64)	138.4 ± 12.3 (N = 64)	156.3 ± 16.0 (N = 64)	138.4 ± 11.5 (N = 64)	156.6 ± 16.2 (N = 64)	137.5 ± 11.6 (N = 64)	155.0 ± 14.0 (N = 64)	136.0 ± 13.0 (N =)
Week 2	206.7 ± 17.2 (N = 64)	163.9 ± 13.0 (N = 64)	207.1 ± 18.1 (N = 64)	163.9 ± 11.9 (N = 64)	206.1 ± 19.1 (N = 63)	162.2 ± 12.9 (N = 64)	201.5 ± 16.2 (N = 64)	160.7 ± 14.8 (N = 64)
adjusted mean	206.8	163.0	206.6	163.0	205.3	162.3	**202.6****	162.3

Table B.6.5.2-2 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 3	260.7 ± 20.1	188.8 ± 15.8	260.0 ± 22.1	187.7 ± 13.7	261.0 ± 21.7	186.6 ± 14.3	252.4 ± 19.7	182.4 ± 16.4
	(N = 64)	(N = 64)	(N = 64)	(N = 64)	(N = 63)	(N = 64)	(N = 64)	(N = 64)
adjusted mean	206.8	187.9	259.4	186.8	206.2	186.6	**253.7****	**184.2****
Week 4	308.4 ± 23.4	207.3 ± 17.1	308.3 ± 26.1	205.9 ± 15.8	309.2 ± 25.6	204.7 ± 15.2	296.3 ± 22.7	198.8 ± 18.6
	(N = 64)	(N = 64)	(N = 64)	(N = 64)	(N = 63)	(N = 64)	(N = 64)	(N = 64)
adjusted mean	308.5	206.4	307.6	204.9	308.4	204.8	**297.7****	**200.7****
Week 5	339.4 ± 25.9	219.0 ± 18.1	342.0 ± 30.2	219.9 ± 15.5	342.0 ± 28.9	216.5 ± 14.8	324.9 ± 27.4	208.8 ± 19.8
	(N = 64)	(N = 64)	(N = 64)	(N = 64)	(N = 63)	(N = 64)	(N = 64)	(N = 64
adjusted mean	339.5	218.1	341.3	219.0	341.1	216.5	**326.5****	**210.6****
Week 6	365.2 ± 29.4	227.3 ± 19.2	365.7 ± 33.9	230.2 ± 16.0	367.8 ± 32.5	223.7 ± 16.6	348.9 ± 30.4	218.7 ± 19.7
	(N = 64)	(N = 64)	(N = 64)	(N = 64)	(N = 63)	(N = 63)	(N = 64)	(N = 64)
adjusted mean	365.3	226.4	364.9	229.3	366.9	223.6	**350.5****	**220.4****
Week 7	388.3 ± 30.0	236.5 ± 18.9	389.7 ± 36.0	236.9 ± 15.4	390.8 ± 35.1	234.1 ± 16.9	369.3 ± 33.2	227.1 ± 20.5
	(N = 64)	(N = 64)	(N = 64)	(N = 64)	(N = 63)	(N = 64)	(N = 63)	(N = 64)
adjusted mean	388.2	235.6	388.7	236.0	389.7	234.1	**370.7****	**228.8****
Week 13	489.4 ± 35.6	266.8 ± 19.5	495.1 ± 44.1	269.3 ± 17.3	494.7 ± 46.8	268.1 ± 17.4	464.9 ± 38.6	258.6 ± 21.8
	(N = 63)	(N = 64)	(N = 64)	(N = 64)	(N = 63)	(N = 63)	(N = 64)	(N = 64)
adjusted mean	488.9	265.9	494.4	268.4	493.6	268.2	**466.8****	**206.4****
Week 51	669.0 ± 54.7	350.9 ± 35.1	676.0 ± 55.9	352.5 ± 31.5	680.1 ± 76.1	350.9 ± 35.4	636.3 ± 53.8	324.2 ± 37.0
	(N = 58)	(N = 64)	(N = 62)	(N = 64)	(N = 62)	(N = 63)	(N = 63)	(N = 63)
adjusted mean	667.8	349.6	677.3	351.2	678.1	350.7	**640.2****	**326.8****
Week 91	627.0 ± 74.2	408.7 ± 36.5	642.7 ± 85.6	420.1 ± 53.5	620.2 ± 90.0	418.2 ± 42.7	626.2 ± 66.3	377.0 ± 43.5
	(N = 30)	(N = 41)	(N = 27)	(N = 42)	(N = 28)	(N = 43)	(N = 37)	(N = 42)
adjusted mean	623.9	410.3	641.2	422.7	617.7	419.9	628.6	**378.8****
Week 104 (males); week 105 (females) adjusted mean	590.6 ± 59.4	390.9 ± 31.8	578.6 ± 85.8	397.2 ± 43.7	572.2 ± 70.6	384.0 ± 48.3	569.6 ± 83.6	363.7 ± 35.7
	(N = 16)	(N = 32)	(N = 17)	(28)	(N = 18)	(N = 39)	(N = 26)	(N = 30)
	592.1	391.0	569.9	398.7	565.0	384.7	560.0	374.7

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

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was lower throughout the first year of the study in animals fed 20000 ppm glyphosate acid. In females the difference was statistically significant over the first 11 weeks (with a maximum reduction of approximately 5%) and again in weeks 40-56 (with a maximum reduction of 6%). In males, the difference was statistically significant over most of the first 6 months with a maximum reduction of 6 %.
The group mean achieved doses are summarised below.

Table B.6.5.2-3Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Group mean achieved dose levels

Dose group	Dietary concentration	Mean achieved dose level (mg/kg bw/day)

	(ppm)	Males	Females
1 (control)	0		
2 (low)	2000	121	145
3 (mid)	6000	361	437
4 (high)	20000	1214	1498

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 121, 361 and 1214 mg/kg bw/day for males and 0, 145, 437 and 1498 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

F. OPHTHALMOSCOPY

There were no treatment-related effects observed.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Minor variations from control values were obtained for most parameters but showed no consistency and were confined to intermediate time points and/or dose groups and were considered not to be treatment-related. An increased haemoglobin concentration and decreased platelet count was seen in all female treated groups at the interim kill while a decrease in Hb was observed at week 14, in the absence of any apparent dose-response or effects at other time points, these variations from mean control values are considered not to be treatment-related (see table below).

Table B.6.5.2-4 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Haemoglobin and platelet count

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)								
Week 14	15.9	15.7	16.0	15.5	16.0	15.9	15.8	**15.0***
Week 27	15.5	15.7	15.8	15.8	15.8	15.7	15.7	15.6
Interim Kill	14.7	14.4	14.4	**15.1****	14.3	**14.9***	14.4	**15.0***
Week 53	16.1	15.9	**15.7***	15.9	**15.5****	15.9	15.9	15.8
Week 79	15.9	15.9	15.2	15.8	15.5	16.0	15.4	15.5
Week 105	13.3	14.3	12.9	14.1	13.1	13.8	13.6	14.2
Platelet count (× 109/L)								
Week 14	885	911	897	877	892	910	847	948
Week 27	903	909	871	868	917	858	880	**830***
Interim Kill	889	821	895	**761***	888	**740****	860	**764***
Week 53	911	842	977	794	911	754	865	814
Week 79	963	854	993	796	950	817	935	855
Week 105	1015	780	980	783	988	750	877	846

* $p < 0.05$; ** $p < 0.01$

Clinical chemistry

In rats fed 20000 ppm glyphosate acid, increases in plasma alkaline phosphatase were present until Week 79 (**Table B.6.5.2-4**). Increases in alanine aminotransferase activities were present consistently in males until Week 79 and in females in Weeks 14, 79 and 105. Increased total bilirubin was also present in these males throughout the study and increased plasma aspartate aminotransferase activity was present in males at the interim kill. Plasma triglycerides and cholesterol levels were reduced (from Weeks 14-53 and Weeks 53 onwards, respectively) in males.

In animals fed 6000 ppm, there were small increases in alkaline phosphatase activity over the first year of the study and variable increases in plasma alanine aminotransferase activity at intermediate time points throughout the study.

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.

In animals, fed 2000 ppm, a slight increase in alkaline phosphatase was observed in week 53 in females (+27%) and at week 79 in males (+22%). In the absence of any other findings this effect was not considered to be adverse.

Other minor variations from mean control values were confined to intermediate dose groups or time points and/or showed no dose response, and so were considered not to be treatment-related.

Table B.6.5.2-4 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Clinical chemical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Alkaline Phosphatase (IU/L)								
Week 14	234	156	246	177	**284** (+20%)	**245** (+57%)	**387** (+65%)	**266** (+71%)
Week 27	196	121	219	136	**239** (+22%)	**166** (+37%)	**327** (+67%)	**203** (+68%)
Interim Kill	230	82	244	102	269	**123*** (+50%)	**306** (+33%)	**144** (+76%)
Week 53	231	92	249	**117***	**277** (+20%)	**152** (+65%)	**357** (+55%)	**172** (+87%)
Week 79	208	114	**254***	131	244	**181** (+59%)	**353** (+70%)	**178** (+56%)
Week 105	184	144	205	129	218	158	280	173
Alanine Aminotransferase (IU/L)								
Week 14	94.9	81.9	103.5	92.5	**121.8** (+28%)	**103.9*** (+27%)	**143.4** (+51%)	**104.7*** (+28%)
Week 27	91.8	99.5	95.9	113.8	116.8	**132.7*** (+33%)	**125.9*** (+37%)	101.8
Interim Kill	77.6	83.4	84.0	82.8	97.7	**113.2*** (+36%)	**123.3** (+59%)	95.9
Week 53	84.2	90.1	99.8	108.2	103.5	**121.5*** (+35%)	**133.8*** (+59%)	114.0
Week 79	69.2	90.0	81.2	97.2	**102.4** (+48%)	110.6	**105.9** (+53%)	**116.0*** (+29%)
Week 105	64.1	83.5	58.6	78.6	63.9	78.9	82.7	**108.2** (+30%)
Total Bilirubin (µmol/L)								
Week 14	1.23	2.00	1.23	1.92	1.46	2.00	**1.85** (+50%)	**2.46*** (+23%)
Week 27	2.08	2.31	2.31	2.08	2.31	2.08	**2.62** (+26%)	2.23
Interim Kill	2.09	2.50	1.91	2.42	2.18	2.58	**2.67** (+28%)	2.64
Week 53	2.62	2.54	2.46	2.31	2.92	2.46	**3.46** (+32%)	**3.15** (+24%)
Week 79	2.46	2.92	2.92	2.31	2.85	2.38	**3.15** (+28%)	3.08
Week 105	1.75	1.19	2.29	1.04	1.67	1.77	2.54	1.40
Aspartate Aminotransferase (IU/L)								

Week 14	107.9	104.5	113.5	112.6	129.2	124.0	**148.0* (+37%)**	114.3
Week 27	110.5	156.8	114.8	185.5	138.0	208.4	141.3	148.3
Interim Kill	90.0	117.8	91.5	109.0	110.4	149.3	**132.0* (+47%)**	131.5
Week 53	111.8	151.9	124.8	194.4	130.2	**219.1* (+44%)**	160.7	**214.8* (+41%)**
Week 79	88.2	156.0	102.7	129.2	130.0	177.7	112.2	197.0
Week 105	75.8	130.7	81.4	102.8	78.4	121.8	92.8	168.5
Plasma Triglycerides (mmol/L)								
Week 14	1.33	1.03	1.48	0.96	1.43	0.96	**1.11* (-17%)**	0.94
Week 27	1.40	1.18	1.42	1.22	1.38	**0.95* (-19%)**	**1.14* (-19%)**	1.09
Interim Kill	1.65	1.00	2.07	1.13	2.09	1.07	1.45	0.99
Week 53	1.53	1.62	1.55	1.75	1.50	1.39	**1.15* (-25%)**	1.39
Week 79	1.90	2.15	1.96	2.77	1.67	2.26	1.42	2.31
Week 105	1.83	3.26	1.81	3.58	1.94	3.02	1.67	2.82
Cholesterol (mmol/L)								
Week 14	2.40	2.66	2.51	2.62	2.48	2.80	2.54	2.71
Week 27	2.92	3.19	3.02	3.24	3.18	3.13	2.98	3.15
Interim Kill	4.74	2.69	5.05	2.95	4.83	2.98	**3.89* (-18%)**	3.01
Week 53	5.03	3.56	4.57	3.49	5.15	3.45	**4.06** (-19%)**	3.66
Week 79	6.87	4.26	6.30	4.64	**5.81* (-15%)**	3.92	**5.20** (-24%)**	3.96
Week 105	6.76	4.44	7.22	4.54	7.79	4.13	**5.72***	4.11
Plasma Creatinine (µmol/L)								
Week 14	58.5	61.4	59.9	59.6	57.2	**59.0***	56.8	**58.6****
Week 27	60.8	62.7	61.2	**60.3***	59.4	**60.5***	**58.4***	**58.2****
Interim Kill	55.8	53.6	58.0	51.8	56.5	52.3	56.6	50.9
Week 53	61.0	58.8	61.5	59.5	62.5	58.1	60.5	58.2
Week 79	80.7	62.7	85.9	59.2	86.2	62.8	66.4	61.8
Week 105	79.1	50.9	80.8	51.4	79.2	53.5	66.2	50.7

* $p < 0.05$; ** $p < 0.01$

H. URINALYSIS

Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate acid (**Table**). Moreover, in the same dose group an increased incidence and severity of blood/red blood cells was present in males and, to a somewhat lesser extent, in females.

There were no other treatment related findings in the urinalysis.

Table B.6.5.2-5 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Urine-analytical findings

	Dose group (ppm)							
	0		**2000**		**6000**		**20000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine pH								
Week 13	6.85	6.00	6.77	6.00	6.92	6.08	**6.31****	5.85

Table B.6.5.2-5 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted], 2001): Urine-analytical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 26	6.77	5.77	6.69	5.85	6.69	6.00	**6.15****	5.77
Week 52	6.85	6.15	6.85	6.23	6.85	6.31	**6.15****	5.92
Week 78	6.54	6.38	6.28	6.77	6.15	6.46	**5.69****	6.00
Week 98	6.08	—	6.00	—	6.00	—	5.85	—
Week 104	—	6.00	—	6.08	—	↑6.15	—	6.00
Blood – week 98/104								
Neg	12	12	13	12	11	12	5	7
Trace	0	0	0	1	0	1	0	0
+	0	0	0	0	0	0	0	2
++	1	1	0	0	2	0	6	4
+++	0	0	0	0	0	0	2	0

** $p < 0.01$

I. NECROPSY

Gross pathology

Treatment-related macroscopic findings were seen in males fed 20000 ppm and/or 6000 ppm in the kidneys, liver, prostate and testes. These findings 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.

Additional findings were not considered to be treatment related.

Organ weights

Significant lower relative adrenal gland weight was noted at the interim kill in females fed 20000 ppm and 6000 ppm glyphosate acid. Furthermore, the liver weight was significantly lower at the interim kill in males fed 20000 ppm glyphosate acid. Significantly reduced the kidney weights were recorded at 6000ppm. The absolute ovary weight at the highest dose group was reduced although the ovary weight relative to body weight did not appear as significantly reduced.

No effect on organ weights was observed in the terminal kill groups.

Table B.6.5.2-6 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Organ weight findings – interim kill

WEEK 53		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		11	11	11	12	12	12	12	11
Adrenal glands	Terminal body weight (g)	682.6	672.9	731.0	627.2	346.5	351.2	344.8	309.5
	Organ weight (g)	0.062	0.057	0.060	0.058	0.077	0.072	**0.068***	**0.063****
	Organ weights adjusted to b.w.	0.062	0.058	0.060	0.058	0.076	0.071	**0.067***	**0.065****
Liver	Terminal body weight (g)	682.6	672.9	731.0	627.2	346.5	351.2	344.8	309.5
	Organ weight (g)	24.4	23.5	25.9	**21.3****	11.4	11.6	11.2	**10.2***
	Organ weights adjusted to b.w.	24.2	23.5	24.4	**22.4***	11.2	11.3	11.0	11.0
Ovaries	Terminal body weight (g)					346.5	351.2	344.8	309.5
	Organ weight (g)	-	-	-	-	0.099	0.092	0.094	**0.078***

Table B.6.5.2-6 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Organ weight findings – interim kill

WEEK 53		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Number of animals		11	11	11	12	12	12	12	11
	Organ weights adjusted to b.w.	-	-	-	-	0.096	0.087	0.092	0.087

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Histopathology
A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate acid at interim and terminal kill (see table below).

Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. The incidence at the top dose was above HCD mean (11.8%) but within HCD range (0-30%; HCD based on 5 studies from the same lab and in the same strain performed between 1998-2003). As the background incidence of hepatitis is highly variable and as the incidence is within HCD range, the relation to treatment is doubted. The incidence of prostatitis was higher than the control group in all treated males and there was a decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm glyphosate acid. The incidence of prostatitis was within historical 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.

The main changes in interim and terminal kill males and, to a lesser extent, females fed 20000 ppm glyphosate acid, were observed in the kidney. These changes consisted of slight increased incidence of papillary necrosis with varying degrees of mineralisation of the papilla and/or transitional cell hyperplasia. There was also a very small increased incidence of papillary mineralisation only (males and females fed 20000 ppm glyphosate acid) and transitional cell hyperplasia alone (20000 ppm males only).

Regarding neoplastic findings, an increased incidence of hepatocellular adenoma was observed in the high dose males (5 versus 0 in control). The study report reported that the incidence at the top dose was not statistically significant using the Fisher's Exact test, however, the difference was statistically significant using the Peto test for trend. The incidence was outside historical control data (refer to Table B.6.5.2-7). However, no effect on non-neoplastic precursors such as hepatocellular hyperplasia was observed. The relevance of the observed hepatocellular adenomas for classification is discussed in the CLH proposal in Volume 1.

All other observed differences in the incidence of histopathological findings either fall within the historical background level or are considered to be unrelated to the treatment with glyphosate acid.

Table B.6.5.2-7 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Summary of histopathological findings

Finding	Dietary concentration of glyphosate (ppm)									
	Males (n=64)					Females (n=64)				
	HCD	0	2000	6000	20000	HCD	0	2000	6000	20000
Liver Proliferative cholangitis	-	56	57	55	64	-	55	58	59	61
Hepatitis	11.8% [0-30%][#]	8 (12.5 %)	6 (9.4 %)	9 (14.1 %)	13 (20.3 %)	-	6	7	4	6
Hepatocellular adenoma [a]	1.5%; [0-5.8%] [#]	0 (0%)	2 (3.1%)	0 (0%)	5 (7.8%)	-	0	0	1	0

Table B.6.5.2-7 Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats ([redacted] 2001): Summary of histopathological findings

Finding	Dietary concentration of glyphosate (ppm)									
	Males (n=64)					Females (n=64)				
	HCD	0	2000	6000	20000	HCD	0	2000	6000	20000
Liposarcoma	-	0	0	1	0	-	0	0	0	0
Kidney Papillary necrosis	0.4 [0 – 2]	0	1	0	14	-	0	1	2	5
Kidney, Transitional cell hyperplasia	-	2	3	0	5	-	3	1	0	1
Kidney, Mineralisation papilla	-	1	2	0	5	-	1	1	0	3
Prostate Prostatitis	23.4 [13 – 35]	13	22	23	37	-	-	-	-	-
Testis Unilateral tubular degeneration	-	18	13	18	5	-	-	-	-	-
Periodontal inflammation	-	25	27	23	42	-	18	24	32	28

n = number of animals per group
Historical control (mean and [range])
5 studies from [redacted] in Alpk:APfsd Wistar BABU performed between 1998-2003.
[a] incidence at the top dose was not statistically significant using the Fisher's Exact test, however, the difference was statistically significant using the Peto test for trend.

Assessment and conclusion by applicant:
In conclusion, glyphosate acid was not carcinogenic in the Wistar rats following continuous dietary exposure of up to 20000 ppm for 24 months (corresponding to 1214 mg/kg bw/day in males and 1498 mg/kg bw/day in females). The NOAEL for toxicity is 6000 ppm (corresponding to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females). In addition, there was no evidence of neurotoxicity.

Assessment and conclusion by RMS:
The proposed NOAEL of 6000 ppm (equal to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females) is agreed with based on the observed clinical chemistry changes and histopathological findings observed in the kidney, liver and prostate.
This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.5.3. Long-term toxicity – rat, study 3

Data point:	CA 5.5/003
Report author	[redacted]
Report year	1997
Report title	Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat
Report No	1231
Document No	Not reported
Guidelines followed in study	OECD 453 (1981)
Deviations from current test guideline (OECD 453, 2018)	The following deviations were noted from the current test guideline:

	• Haematology was performed without determining haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time, platelet count; • clinical chemistry was performed without determining inorganic phosphorous, calcium, chloride, sodium, potassium, cholesterol, creatinine • Urinalysis did not include volume, osmolality or specific gravity. • organ weights were not determined for all animals; weights of epididymides, heart, spleen, (para)thyroids and uterus were not determined. In addition, for the main group the organs of only 10 animals were weighted • histopathology was performed without determining Harderian gland, cervix, coagulating gland, lacrimal gland, vagina. In addition, for the mid and high dose group only a small number of animals were examined histopathologically except for liver, kidneys, lungs, testes, adrenals and ovaries for which all animals were examined.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant :** Invalid (Category 3b): - Information on test substance identification not available and several deviations from the current OECD TG 453 evident. **Conclusion AGG :** A number of major deviations/limitations were noted : • Test substance is unclear as no batch number nor purity is provided. No storage conditions or expiry data was provided. • Several parameters which are required under OECD 453 were not included. • The reported incidence of neoplasia in the controls is unusually low, e.g. in control males only two incidences in total is reported (one seminoma in the testes and one fibroadenoma in the mammary gland) Based on these limitations the study is concluded to be unacceptable.

Full summary

The chronic toxicity and carcinogenic potential of Glyphosate technical was assessed in a 24-month feeding study in male and female Sprague Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 3000, 15000, and 25000 ppm Glyphosate technical (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)).
In addition 20 rats/sex/group were included for interim sacrifice at Week 52, to study non-neoplastic histopathological changes with a different high dose level of 30000 ppm. 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.
Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on food consumption noted. Significantly reduced body weight gain that lasted throughout study until termination was observed in males receiving the highest dose (overall body weight gain reduced by 12%). In all other groups body weight gain was comparable to the control at termination. Apart from increased alkaline phosphatase levels in the high dose of the carcinogenicity study at study termination, all other

significant changes observed in haematological, biochemical and physio-pathological parameters of urine lacked a dose-response relationship, showed inconsistent increases and decreases and/or were only observed at one timepoint and hence appear to be of no biological significance.

Gross pathology and histopathological examination revealed no treatment-related and dose-dependent effects. Regarding organ weights, significant and dose-dependent effects after 52 weeks were found only in animals dosed at 30000 ppm consisting of increased relative brain and testis weight likely due to the decrease in body weight as well as increased relative kidney weight and relative liver weight (females only).
In the carcinogenicity study which lasted 52 weeks longer, significant and dose-dependent effects in males consisted of increased relative weight of brain and testes in the mid and high dose group likely due to the decreased body weight. Effects on the kidneys were not observed, perhaps due to the lower dose level in the highest group compared to the chronic toxicity study, e.g. 25000 ppm to 30000 ppm, respectively. In females, significant and dose-dependent effects after 24 months occurred only in kidneys.

In conclusion, glyphosate technical was not carcinogenic in the Sprague Dawley rats following continuous dietary exposure of up to 1.29 g/kg bw/day for males and 1.74 g/kg bw/day for females for 24 months. The NOAEL is concluded to be 15000 ppm (corresponding to 780 mg/kg bw/day in males and 1060 mg/kg bw/day in females) based on the increase in ALP in both sexes and increased kidney weight in females .

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	No data given in the report
Lot/Batch #:	No data given in the report
Purity:	No data given in the report
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control:

Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 6 weeks
Sex:	Males and females
Weight at dosing:	Males: 70.0 – 93.2 g, females: 70.0 – 90.6 g
Acclimation period:	One week
Diet/Food:	Powdered rat feed (Lipton India Ltd, India), *ad libitum*
Water:	Filtered pure water, *ad libitum*
Housing:	Initially in groups of five in polypropylene cages, in groups of three from Week 24 to 52 and in groups of two from Week 53 to termination.
Environmental conditions:	Temperature: 22 - 25 °C Humidity: 50 - 70 % Air changes: 10 - 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-06-09 to 1996-06-12

Animal assignment and treatment:
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 (equivalent to mean achieved dose levels of 0, 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0, 0.21, 1.06 and 1.74 g/kg bw/day (females)) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex were 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.

Clinical observations
Rats were examined for toxic signs once and pre-terminal deaths twice a day. Ophthalmic examination was done at the start of the study, at interim sacrifice and at termination in the control and high dose group.

Body weight
Individual body weights were recorded on Day 0, at weekly intervals thereafter until the end of Week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each group from Week 1 to Week 13 and subsequently in Week 25, 38, 51, 65, 78, 92 and 104.

Haematology and clinical chemistry
Haematology
Individual blood samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Haemoglobin, erythrocyte count, PCV, thrombocytes, total leukocyte count and differential leukocyte count.

Blood chemistry
Individual plasma samples were collected from 10 rats/sex/group of the main groups at 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. Before sampling animals were fasted overnight. The following parameters were measured: Total serum proteins, albumin, ALT, AST, GGTP, SAP, blood urea nitrogen and blood glucose.

Urinalysis
Individual urine samples were collected from 20 rats/sex/group of the main groups at 3, 6, 12, 18 and 24 months and from all surviving animals of the satellite group at 12 months. The following measurements were made: Specific gravity, volume, appearance, pH, protein, glucose, occult blood, ketones, microscopy of sediments.

Sacrifice and pathology
Necropsy was performed on all animals at scheduled termination.

The following organ weights were determined from 10 rats per sex per main group and on all animals of the satellite groups: adrenals, brain, gonads, kidneys and liver.

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.

Tissue samples were taken from the following organs of all animals: adrenals, aorta, body cavities, brain, caecum, colon, duodenum, epididymides, eyes (both), femur, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate,

rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum with bone marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder and uterus.

Statistics
Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958). Animals withdrawn from study during the interval (those taken for moribund sacrifice) are taken into consideration by giving enough weightage.

The incidence of neoplasms was analysed by Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.

In addition to these tests the Fisher exact test for pairwise comparisons and the Cochran Armitage linear trend test for dose response trends were carried out. All reported P-values for the tumour incidence analysis are one-sided.

The biochemical, haematological and organ weight data was analyzed for significance using Student 't' test or Cochran 't' test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analyses for concentrations showed that the diet preparations recovered 86.1 - 98.3 % of the target concentration. Analyses for homogeneity recovered 87.5 - 90.0 % for 3000 ppm, 91.7 - 93.0 % for 15000 ppm, 94.3 - 95.1 % for 25000 ppm and 91.8 - 92.6 % for 30000 ppm.
Stability analyses showed that recovery one month after diet preparation ranged between 87.5 and 95.0 %.

B. MORTALITY
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.

The numbers of pre-terminal deaths in the main group are displayed in the table below:

Table B.6.5.3-1 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Cumulated mortalities after 104-week dietary exposure to Glyphosate technical*

Sex	Dose group (ppm)			
	0	3000	15000	25000
Male	16/50	17/50 (2)	18/50 (4)	23/50 (14)
Female	19/50	20/50 (2)	20/50 (2)	25/50 (12)

* Values in parentheses indicate increases in mortality compared to control in percent.

C. CLINICAL OBSERVATIONS
No significant toxic signs were observed in treated or control groups.

D. BODY WEIGHT
Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose (-12%). In all other groups body weight gain was comparable to the control at termination. At 104 weeks the reduction of body weight was 8% in males of the satellite group and 9.8% in males of the main group. The effect on body weight was not considered adverse as the decrease of body weight was below 10%.

Table B.6.5.3-2 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Group mean body weights (satellite group)

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	3000	15000	30000	0	3000	15000	30000
	No. of rats	20	20	20	20	20	20	20	20
Week 0		80.27 ± 5.97	78.45 ± 5.91	78.74 ± 5.20	81.09 ± 5.23	78.83 ± 4.88	77.56 ± 5.39	77.01 ± 5.91	77.83 ± 4.97
Week 1		106.20 ± 7.45	102.96 ± 6.89	101.15 ± 5.36	102.76 ± 7.08	97.22 ± 4.62	99.69 ± 4.03	98.75 ± 5.05	99.70 ± 4.31
Week 2		134.25 ± 10.67	132.56 ± 9.27	128.79 ± 11.39	132.95 ± 11.55	121.10 ± 6.93	125.20 ± 5.72	125.55 ± 7.17	125.91 ± 6.94
Week 3		163.89 ± 17.06	161.39 ± 11.78	160.42 ± 9.57	160.36 ± 14.26	143.97 ± 8.36	150.44 ± 7.39	150.43 ± 8.10	149.81 ± 7.62
Week 13		297.01 ± 30.02	295.50 ± 21.82	278.93 ± 21.44	266.65 ± 27.71	212.94 ± 17.66	212.78 ± 11.58	221.59 ± 12.82	203.96 ± 13.33
Week 52		408.89 ± 31.75	409.23 ± 32.55	389.42 ± 31.63	384.32 ± 38.06	268.24 ± 28.29	253.65 ± 16.80	268.85 ± 23.12	259.47 ± 24.17

Table B.6.5.3-3: Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Group mean body weights (main group)

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	3000	15000	30000	0	3000	15000	30000
	No. of rats	20	20	20	20	20	20	20	20
Week 0		80.27 ± 6.22	80.68 ± 5.99	78.69 ± 5.65	80.01 ± 4.91	79.69 ± 5.77	79.34 ± 5.10	78.15 ± 4.87	77.98 ± 4.84
Week 1		103.52 ± 7.88	104.03 ± 7.71	104.33 ± 7.49	105.15 ± 7.18	101.02 ± 5.38	101.13 ± 5.60	101.56 ± 6.01	101.64 ± 6.31
Week 2		133.72 ± 12.36	134.29 ± 9.33	133.72 ± 9.12	132.34 ± 8.12	127.92 ± 7.44	129.04 ± 5.87	126.37 ± 7.40	126.71 ± 7.48
Week 3		163.95 ± 15.44	164.20 ± 11.55	164.26 ± 8.25	162.92 ± 8.70	155.85 ± 9.13	157.37 ± 6.64	154.25 ± 7.93	155.05 ± 8.73
Week 13		296.67 ± 31.82	285.08 ± 18.81	277.94 ± 24.92	270.84 ± 14.61	217.42 ± 17.46	213.09 ± 22.21	203.41 ± 15.10	206.27 ± 15.56
Week 53		403.19 ± 31.75	398.15 ± 32.55	384.34 ± 33.76	374.76 ± 29.85	280.45 ± 36.54	269.18 ± 29.30	257.82 ± 23.69	261.64 ± 26.85
Week 104		406.05 ± 61.32	385.51 ± 57.94	391.09 ± 55.86	366.58 ± 47.54	313.72 ± 58.93	315.60 ± 58.03	288.13 ± 42.77	284.52 ± 43.31

E. FOOD CONSUMPTION AND COMPOUND INTAKE

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.

The group mean achieved doses are summarised below.

Table B.6.5.3-4 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (g/kg bw/day)*	
		Males	Females
		Chronic toxicity study (52 weeks)	
low	3000	0.18	0.24
mid	15000	0.92	1.13
high	30000	1.92	2.54
		Carcinogenicity study (104 weeks)	
low	3000	0.15	0.21
mid	15000	0.78	1.06
high	25000	1.29	1.74

* Calculations were done with values from Week 13 (chronic) and Week 25 (carcinogenicity)

F. OPHTHALMOLOGICAL EXAMINATION

Ophthalmological examinations revealed no abnormalities.

G. LABORATORY INVESTIGATION

Haematological examination did not reveal any abnormalities attributable to the treatment.

Regarding the clinical chemical investigations, a significant increase in the alkaline phosphatase level seen in the high dose of the carcinogenicity study at study termination in both males and females (see table below). A statistical increase was also observed at 24 months in the mid dose males. However, considering that the effect is only slight without any concomitant liver findings it is not considered to be adverse.
At the 12 month time point a slight statistically significant increase was observed at all dose levels in females. However, this effect lacked a dose response.

Other significant changes observed in haematological, and biochemical parameters lacked a dose-response relationship, showed inconsistent increases and decreases and/or were only observed at one timepoint and hence appear to be of no biological significance.

Table B.6.5.3-5 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Statistically significant changes in blood chemistry

	Dose group (ppm)							
	Males				Females			
	0	**3000**	**15000**	**25000**	**0**	**3000**	**15000**	**25000**
Alkaline phosphatase								
Month 6	25.58 ± 3.20	24.97 ± 3.61	24.85 ± 3.77	23.07 ± 2.75	24.96 ± 3.22	25.25 ± 3.70	25.2 ± 3.26	25.11 ± 3.71
Month 12	25.64 ± 5.28	25.96 ± 3.84	27.64 ± 3.65	22.88 ± 3.12	19.04 ± 2.87	**25.35* ± 3.62**	**28.3* ± 3.61**	**22.88* ± 3.72**
Month 18	27.7 ± 4.55	25.94 ± 3.42	28.73 ± 2.89	26.68 ± 3.85	24.47 ± 5.56	28.42 ± 3.97	27.71 ± 3.84	25.28 ± 2.32
Month 24	26.04 ± 4.96	26.75 ± 4.22	**28.42* ± 4.57**	**47.71* ± 5.70**	24.87 ± 4.19	**26.95*** ± 3.00	25.75 ± 4.25	**53.86* ±** 5.49

*= $p \leq 0.05$

H. URINALYSIS

Urinalysis did not reveal any abnormalities attributable to the treatment.

I. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed during the study period.

Organ weights
In high dose males in the interim group, increased relative weights of kidneys, brain and testes were observed. The increase in relative brain and testis weight are likely due to the decreased body weight. In females, in addition to relative kidneys and brain, the relative liver weight was increased as well (+10%).

In the carcinogenicity study which lasted 52 weeks longer, significant and dose-dependent effects in males consisted of increased relative weight of brain and testes in the mid and high dose group likely due to the decreased body weight. Effects on the kidneys were not observed, perhaps due to the lower dose level in the highest group compared to the chronic toxicity study, e.g. 25000 ppm to 30000 ppm, respectively.

In females, significant and dose-dependent effects after 24 months occurred only in kidneys. Like for male animals, this increase could be due to the different high dose levels.

Table B.6.5.3-6 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Organ weights

mg/kg bw/d		Body Weight (g)	Liver (g)	Kidneys (g)	Brain (g)	Adrenals (g)	Testes (g)	Ovaries (g)
Satellite group (males) absolute values	0	393.70 ± 31.39	14.17 ± 1.73	3.16 ± 0.34	2.24 ± 0.11	0.067 ± 0.013	3.43 ± 0.30	-
	3000	393.07 ± 31.63	13.76 ± 1.75	3.01 ± 0.29	**2.09* ± 0.26**	0.059 ± 0.014	3.38 ± 0.35	-
	15000	372.79 ± 30.85	13.02 ± 1.92	2.98 ± 0.36	2.22 ± 0.17	0.058 ± 0.01	**3.01* ± 0.53**	-
	30000	369.52 ± 34.53	14.35 ± 2.80	3.20 ± 0.43	2.31 ± 0.12	0.059 ± 0.014	3.47 ± 0.41	-
Satellite group (males) relative values	0	393.69 ± 31.39	3.602 ± 0.363	0.805 ± 0.075	0.573 ± 0.048	0.017 ± 0.004	0.874 ± 0.087	-
	3000	393.08 ± 31.63	3.507 ± 0.416	0.768 ± 0.080	0.536 ± 0.081	0.015 ± 0.004	0.866 ± 0.119	-
	15000	372.76 ± 30.85	3.525 ± 0.668	0.806 ± 0.123	0.600 ± 0.068	0.058 ± 0.010	0.812 ± 0.151	-
	30000	369.52 ± 34.53	3.863 ± 0.450	**0.866* ± 0.088**	**0.629* ± 0.064**	0.016 ± 0.004	**0.943* ± 0.098**	-
Satellite group (females) absolute values	0	258.97 ± 26.83	8.63 ± 1.45	1.88 ± 0.24	1.94 ± 0.15	0.068 ± 0.014	-	0.115 ± 0.036
	3000	244.31 ± 14.87	7.89 ± 0.78	1.84 ± 0.09	2.00 ± 0.11	**0.053* ± 0.012**	-	0.121 ± 0.120
	15000	259.13 ± 21.71	8.15 ± 0.96	1.90 ± 0.14	2.06 ± 0.12	**0.060* ± 0.009**	-	0.133 ± 0.160
	30000	252.84 ± 23.56	9.25 ± 1.42	1.98 ± 0.16	**2.08* ± 0.11**	0.061 ± 0.01	-	0.123 ± 0.071
Satellite group (females) relative values	0	258.97 ± 26.93	3.324 ± 0.412	0.726 ± 0.070	0.756 ± 0.098	0.026 ± 0.006	-	0.045 ± 0.015
	3000	244.31 ± 14.87	3.229 ± 0.270	0.756 ± 0.047	**0.819* ± 0.045**	**0.022* ± 0.006**	-	0.049 ± 0.046
	15000	259.13 ± 21.70	3.163 ± 0.425	0.737 ± 0.057	0.799 ± 0.065	0.023 ± 0.004	-	0.037 ± 0.009
	30000	252.84 ± 23.56	**3.662* ± 0.459**	**0.788* ± 0.073**	**0.828* ± 0.067**	0.024 ± 0.006	-	0.049 ± 0.029
Main group (males)	0	445.17 ± 31.44	19.92 ± 2.46	3.91 ± 0.34	2.28 ± 0.13	0.080 ± 0.019	3.46 ± 0.55	-
	3000	422.03 ± 34.94	17.32 ± 3.88	3.41 ± 0.83	2.25 ± 0.14	0.089 ± 0.007	3.45 ± 1.16	-

Table B.6.5.3-6 Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat ([redacted] 1997): Organ weights

mg/kg bw/d		Body Weight (g)	Liver (g)	Kidneys (g)	Brain (g)	Adrenals (g)	Testes (g)	Ovaries (g)
absolute values	15000	397.69 ± 35.94	**16.00* ± 2.50**	3.48 ± 0.35	2.28 ± 0.14	0.077 ± 0.017	3.91 ± 0.83	-
	25000[1]	379.14 ± 19.21	**16.08* ± 2.52**	**3.36* ± 0.34**	2.34 ± 0.16	0.069 ± 0.006	3.65 ± 0.45	-
Main group (males) relative values	0	445.17 ± 31.44	4.45 ± 0.66	0.88 ± 0.07	0.51 ± 0.06	0.018 ± 0.004	0.78 ± 0.13	-
	3000	422.03 ± 34.94	4.13 ± 1.00	0.81 ± 0.20	0.54 ± 0.05	0.021 ± 0.002	0.81 ± 0.25	-
	15000	397.69 ± 35.94	4.05 ± 0.69	0.88 ± 0.07	**0.58* ± 0.058**	0.019 ± 0.004	**0.99* ± 0.24**	-
	25000[1]	379.14 ± 19.21	4.25 ± 0.68	0.89 ± 0.10	**0.62* ± 0.06**	0.018 ± 0.002	**0.97* ± 0.12**	-
Main group (females) absolute values	0	319.12 ± 32.62	14.06 ± 2.78	2.53 ± 0.36	2.18 ± 0.13	0.099 ± 0.009	-	0.174 ± 0.07
	3000	318.83 ± 41.15	12.00 ± 1.76	2.64 ± 0.10	**2.28* ± 0.04**	0.091 ± 0.009	-	0.180 ± 0.03
	15000	300.95 ± 13.72	**11.23* ± 1.01**	2.40 ± 0.12	2.19 ± 0.06	**0.076* ± 0.01**	-	0.125 ± 0.07
	25000	286.38 ± 38.69	12.65 ± 2.05	2.61 ± 0.39	2.10 ± 0.08	0.085 ± 0.023	-	0.187 ± 0.060
Main group (females) relative values	0	319.12 ± 32.62	4.42 ± 0.77	0.79 ± 0.10	0.69 ± 0.07	0.031 ± 0.004	-	0.056 ± 0.025
	3000	318.83 ± 41.15	**3.79* ± 0.52**	0.84 ± 0.07	0.73 ± 0.08	0.029 ± 0.003	-	0.057 ± 0.01
	15000	300.95 ± 13.72	**3.74* ± 0.43**	0.80 ± 0.07	0.73 ± 0.04	**0.025* ± 0.003**	-	0.042 ± 0.002
	25000	286.38 ± 38.69	4.49 ± 0.96	**0.92* ± 0.14**	0.76 ± 0.11	0.030 ± 0.008	-	0.066 ± 0.026

*= $p \leq 0.05$

[1] Note AGG : A mistake is noted in the study report. Two different values are provided for the terminal body weight of high dose males at 24 months, namely 379.14 g and 379.40 g. Based on the individual animal data the correct value is 379.14 g. The difference is only minor but does add to the concern regarding the reliability of the study.

Histopathology

Histopathological changes were found at all dose levels including control. Based on histopathology observation no treatment-related effects were observed.

Neoplastic changes

There were no treatment-related neoplasms observed.

Assessment and conclusion by applicant:

Based on the 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 month is 25000 ppm (corresponding to 1290 mg/kg bw/day for males and 1740 mg/kg bw/day for females). It is concluded that Glyphosate technical is not carcinogenic in rats.

Based on the deviations such as lack of information on test substance identification, storage conditions and several deviations from the current OECD TG 453 the study is not considered acceptable for hazard and risk assessment. Drawing any reliable conclusion concerning a NOAEL from the study is not possible.

Assessment and conclusion by RMS:

Based on the increase in ALP (+83% in males, +117% in females) and increased kidney weight in females the NOAEL is concluded to be 15000 ppm (corresponding to 780 mg/kg bw/day in males and 1060 mg/kg bw/day in females).

It is noted that in the original RAR the NOAEL was set at 3000 ppm based on clinical chemistry parameters without concomitant liver pathology arguing that a lower number of animals were examined histopathologically in the low and high dose. However, the AGG notes that for the liver, kidneys, lungs, testes, adrenals and ovaries all animals were tested and therefore considers the NOAEL of 15000 ppm to be justified.

Based on the deviations from OECD453, the lack of information on the test substance tested and the unusual low background incidence of neoplasia in the controls the study is concluded to be unacceptable.

B.6.5.4. Long-term toxicity – rat, study 4

Data point:	CA 5.5/004
Report author	[redacted]
Report year	1997
Report title	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats
Report No	[redacted]94-0150
Document No	NA
Guidelines followed in study	OECD 453 (1981), JMAFF 59 NohSan 3850 (1984), US-EPA (1989)
Deviations from current test guideline (OECD 453, 2018)	The following deviations from the current OECD guideline were noted : - prothrombin time and activated partial thromboplastin time were not investigated; - organ weights of epididymides, heart, ovaries, spleen, uterus and (para)thyroid were not measured; lacrimal gland were not investigated histopathologically.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid, Category 2a **Conclusion AGG:** Some minor deviations were noted compared to the current OECD test guideline. However, these are not considered to be critical and therefore the study is considered to be acceptable.

Full summary

The chronic toxicity and carcinogenic potential of HR-001 (Glyphosate technical) was assessed in a 24-month feeding study in male and female Sprague-Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 3000, 10000, and 30000 ppm HR-001 (equal to 0, 104, 354 and 1127 mg/kg bw/day for males and 0, 115, 393 and 1247 mg/kg bw/day for females). In addition, 30 rats/sex/group were included for interim sacrifice at 26, 52 and 78 weeks to study non-neoplastic histopathological changes. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths in any of the dose-groups. Clinical observations consisted of loose faeces together with soiled fur in the perianal region in the high dose group as well as increased incidences of tail mass in the mid and high dose group. Moreover, decreases in body weight were observed in both sexes in the mid and high dose group along with a lower food consumption although the effect in the mid dose were only slight and not

considered to be adverse. Ophthalmological examinations, urinalysis and haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.

Necropsy supported the clinical signs of loose stool by increased incidences of distension of the caecum in the mid and high dose group together with increased absolute and relative caecum weights in the mid and high dose group. Moreover, the increased incidences of thickened areas in the skin of the tail, corresponding to the increased incidences of tail mass, were histopathologically diagnosed as follicular hyperkeratosis in the mid and high dose group. Skin keratoacanthoma was observed in the high dose group.

The NOAEL was concluded to be 3000 ppm, equal to 104 mg/kg bw/day for males and 115 mg/kg bw/day for females).

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209; T-950308
Purity:	97.56 %; 94.61 %
Stability of test compound:	No data given the report.
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crj:CD)
Source:	[redacted]
Age:	5 weeks (males), 6 weeks (females)
Sex:	Males and females
Weight at dosing:	65 – 85 g
Acclimation period:	At least one week
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd, Japan), *ad libitum*
Water:	Well water treated with sand and charcoal filter, HCl and UV rays, *ad libitum*
Housing:	In groups of ten animals of the same sex in wire-mesh stainless steel cages during the acclimatisation period. During the study males were housed in groups of 5 per cage until week 72, in groups of ≤3 until week 78 and individually thereafter. Females were housed in groups of five until week 78, and individually thereafter.
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-12-19 to 1996-12-25

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenficity study groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000 and 30000 ppm (equivalent to mean achieved dose levels of 0, 104.7, 354.0 and 1127.0 mg/kg bw/day in males and 0, 114.7, 393.0 and 1247.0 mg/kg bw/day in females) HR-001. In addition, 30 rats/sex/group were included for interim sacrifices at 26, 52 and 78 weeks.

Test diets were prepared weekly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to a larger amount of basal diet and blended by a blending machine.
The stability of the test substance in food was previously determined in a 4-week dose-range finding study in mice. Homogeneity analyses were performed on samples of each dose level of the first diet preparation. Analyses for achieved concentrations were done for each dose level in monthly intervals.

Observations
Rats of all groups were examined for toxic signs and pre-terminal deaths once a day. In addition a detailed veterinary examination was made at least once per week. Ophthalmic examination was done at the start of the study and at termination.

Body weight
Individual body weights were recorded at weekly intervals until the end of Week 13 and every 4 weeks thereafter and before necropsy, except for dead or moribund satellite animals, which were discarded without body weight determination.

Food consumption and compound intake
Food consumption was measured for a period of three consecutive days weekly from Week 1 to 13 and every four weeks from Week 16 to 104. Mean individual food consumption, group mean food consumption and group compound intake were calculated.

Haematology and clinical chemistry
Blood samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in week 104. Before sampling animals were fasted overnight. The following parameters were measured: hematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, alkaline phosphatase (ALP), glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), γ-glutamyl-transpeptidase, creatine phosphokinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, albumin/globulin ratio, total bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from 10 rats/sex/group of the satellite groups in Week 26, 52, from all surviving animals of the satellite group in Week 78 and from 10 rats/sex/group of the main group in Week 104. The following measurements were made: density, volume, appearance, pH, protein, glucose, occult blood, ketones, urobilinogen, sediments.

Sacrifice and pathology
Necropsy and histopathological examinations were carried out on all tissues collected at interim and terminal sacrifice. The following organ weights were determined from all animals: adrenals, brain, caecum, kidneys, liver and testis.

Tissue samples were taken from the following organs: adrenals, aorta, bone & bone marrow (sternum and femur incl. joint), brain (cerebrum, cerebellum, pons and medulla oblongata), caecum, colon, duodenum, epididymides, eyes, gross lesions, Harderian glands, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands (submaxillary and sublingual), sciatic nerve, seminal vesicles and coagulating glands, skeletal muscle, skin (females only), spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix) and vagina.

Statistics
Statistical significance of the difference between the control group and the treated groups was estimated at 5 % and 1 % levels of probability.

The data of body weight (main group only), food consumption, urine specific gravity, urine volume, haematological parameters, blood biochemical parameters, and organ weights were evaluated by Bartlett's test for equality of variance. When group variances were homogeneous, a parametric analysis of variance of a one-way layout type was conducted to determine if any statistical differences existed among groups. When the analysis of variance was significant, Dunnett's (when sample size of each group was equal) or Scheffé's (when sample size of each group was different) multiple comparison test was applied to evaluate differences between the treated and the control groups. When the group variances were heterogeneous, the data were analyzed by Kruskal-Wallis non-parametric analysis of variance. When significant, Dunnett type (when sample size of each group was equal) or Scheffé type (when sample size of each group was different) mean rank sum test was applied to determine if any significant differences existed between the treated and the control groups.

The data of urinalysis except for specific gravity and urine volume were assessed by Mann-Whitney's U test.

Mortality was analysed by Life table analysis.

The data of clinical sign (main group only), ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

The coefficient of variation for the homogeneity of the test substance for each dose level was 2.2% and less. Hence, the results indicated a good homogeneity.

Analyses for concentrations showed that the diet preparations achieved 97 - 98% of the target concentration. Thus, the concentrations of the test substance in the test diets were within acceptable limits.

B. MORTALITY AND CLINICAL SIGNS

In the high dose group neither sex showed an increase in mortality. 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. The final mortality is given in Table B.6.5.4.1:

Table B.6.5.4-1 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Final mortality at termination of treatment (%)

Sex	Dose group (ppm)*			
	0	3000	10000	30000
Male	32/50 (64)	30/50 (60)	32/50 (64)	21/50 (42)
Female	35/50 (70)	31/50 (62)	34/50 (68)	36/50 (72)

* number of mortalities / total number of rats/group (% mortality)

C. CLINICAL OBSERVATIONS

In the high dose group, significant increases in incidence of bradypnea, mass, 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 high in incidence compared to 11 of the control. The incidences of mass 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 significant 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. Animals showing loose stool could not be identified because of group housing, therefore the sign is only described here in the text but not included in the table below.

In the mid dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control which lacked a dose response relationship.

In the low dose group, significant increases in incidence of decreased spontaneous motor activity, bradypnea, and soiled fur and a significant 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. It is noted that no clear dose-relationship is observed for these findings.

Table B.6.5.4-2 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of changes in clinical signs

Conditions	Sex	Males				Females			
	Group #	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
	Dose (ppm)	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
	No. of rats	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Appearance: Emaciation		12	16	14	14	23	28	18	26
Posture: Posterior paralysis		0	0	1	0	-	-	-	-
Behaviour: Decreased spontaneous motor activity		9	**19***	9	13	23	22	20	26
Respiration: Bradypnea		3	**10***	4	**11***	7	14	6	12
Periocular region: Ptosis		7	6	4	6	4	**12***	6	6
Perinasal region: Tactile hair loss		5	**0***	**0***	**0***	1	**17****	**9****	4
Integument: Wound		7	4	6	0**	2	2	2	1
Integument: Mass		22	26	21	**37****	37	36	38	43
Integument: Hair loss		12	7	15	**3***	16	13	21	25
Integument: Soiled fur		10	**20***	12	**21***	16	17	11	18
Integument: Wetted fur		9	7	7	16	5	5	5	**15***

* Statistically significant ($p \leq 0.05$); ** Statistically significant ($p \leq 0.01$)

D. BODY WEIGHT

In the high dose group, body weights were lower than the control throughout the treatment period; significant decreases in their body weights were observed during Weeks 1 to 80 in males and at Week 7 and during Weeks 9 to 60 in females. The final group mean body weights of males and females at termination of the treatment period were both 93% of the respective control.

In the mid dose group, males showed a minor decrease in body weight gain during the first few weeks of treatment with a statistically significant difference from the control at Week 6. Their retarded growth persisted throughout the treatment period, and the final group mean body weight at termination of treatment was 95% of the control. Body weight change in mid dose females was comparable to the control throughout the treatment period.

In the low dose group, body weights of both sexes were comparable to the control except for a significant increase in females at Week 16.

Table B.6.5.4-3 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of body weight (g)

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	50	50	50	50	50	50	50	50
Week 0		151 ± 8	151 ± 8	151 ± 8	151 ± 8	116 ± 6	116 ± 6	116 ± 6	116 ± 6
Week 1		213 ± 12	213 ± 12	212 ± 13	**205** ± 12 (-4%)**	153 ± 10	153 ± 9	154 ± 8	150 ± 7
Week 2		275 ± 17	275 ± 17	271 ± 17	**261** ± 16 (-5%)**	179 ± 12	179 ± 12	178 ± 10	175 ± 10
Week 3		329 ± 20	326 ± 21	324 ± 20	**310** ± 21 (-6%)**	205 ± 15	206 ± 16	205 ± 13	201 ± 13
Week 4		371 ± 24	369 ± 24	362 ± 24	**345** ± 24 (-7%)**	226 ± 16	229 ± 18	228 ± 15	222 ± 15
Week 5		409 ± 28	407 ± 27	396 ± 30	**378** ± 28 (-8%)**	245 ± 18	250 ± 22	247 ± 19	238 ± 17
Week 6		439 ± 31	437 ± 31	**422* ± 41**	**408** ± 32 (-7%)**	260 ± 19	265 ± 23	261 ± 19	252 ± 18
Week 7		464 ± 34	462 ± 32	447 ± 44	**431** ± 34 (-7%)**	273 ± 20	280 ± 25	275 ± 20	**262* ± 18 (-4%)**
Week 8		484 ± 35	479 ± 34	467 ± 39	**447** ± 37 (-8%)**	280 ± 21	288 ± 26	283 ± 22	270 ± 19
Week 9		501 ± 37	496 ± 35	486 ± 41	**459** ± 38 (-8%)**	289 ± 21	298 ± 27	292 ± 22	**277* ± 21 (-4%)**
Week 10		520 ± 38	515 ± 38	502 ± 43	**474** ± 40 (-9%)**	296 ± 22	304 ± 28	297 ± 24	**282* ± 21 (-5%)**
Week 11		536 ± 39	532 ± 40	518 ± 46	**487** ± 41 (-9%)**	302 ± 22	312 ± 30	305 ± 24	**288* ± 21 (-5%)**
Week 12		549 ± 41	544 ± 43	530 ± 46	**499** ± 44 (-9%)**	310 ± 23	322 ± 32	312 ± 25	**294** ± 23 (-5%)**
Week 13		562 ± 43	557 ± 46	545 ± 48	**511** ± 45 (-9%)**	316 ± 24	328 ± 33	317 ± 27	**297** ± 23 (-6%)**
Week 80		840 ± 112	843 ± 116	828 ± 106	**770* ± 91 (-8%)**	525 ± 94	558 ± 93	544 ± 104	503 ± 70
Week 104		811 ± 114	800 ± 108	772 ± 172	758 ± 117	558 ± 119	521 ± 128	545 ± 118	517 ± 142

E. Food consumption and compound intake

In the high dose group, consistent with the decreasing body weight or decreasing body weight trends, food consumption showed a decreasing trend in males during the first few weeks. After the initial few weeks no differences were noted.

In the other groups, food consumption in males and females was comparable to the respective control.

Table B.6.5.4-4 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of food consumption data (g)

Timepoint	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	50	50	50	50	50	50	50	50
Week 1		16.7 ± 1.7	16.7 ± 0.8	16.6 ± 0.3	**14.7** ± 1.0**	13.0 ± 0.4	13.4 ± 0.5	**13.5* ± 0.3**	12.5 ± 0.5
Week 2		20.1 ± 0.9	20.4 ± 0.8	20.4 ± 0.6	19.9 ± 0.8	15.0 ± 0.8	14.6 ± 0.6	14.7 ± 0.6	14.7 ± 0.5
Week 3		23.0 ± 0.7	22.9 ± 0.8	22.4 ± 0.6	22.2 ± 0.9	15.3 ± 0.8	15.9 ± 0.7	15.8 ± 0.8	15.7 ± 0.4
Week 4		23.6 ± 0.7	22.9 ± 0.9	**22.6* ± 0.4**	**22.4** ± 1.1**	16.0 ± 0.8	16.2 ± 1.2	16.4 ± 0.9	15.8 ± 0.8
Week 5		22.9 ± 1.3	22.9 ± 1.0	22.5 ± 0.9	21.9 ± 1.2	17.1 ± 0.8	17.4 ± 0.9	16.6 ± 0.7	**16.1* ± 1.0**
Week 6		23.0 ± 1.3	23.3 ± 1.1	22.6 ± 1.7	22.7 ± 1.4	16.8 ± 0.8	17.5 ± 1.0	17.3 ± 0.8	16.2 ± 0.6
Week 13		24.2 ± 1.2	23.9 ± 1.4	23.6 ± 0.8	23.4 ± 1.0	15.7 ± 0.8	16.3 ± 0.8	15.5 ± 0.8	15.2 ± 0.9
Week 80		26.0 ± 2.1	25.6 ± 2.0	25.7 ± 1.7	25.4 ± 1.7	16.0 ± 1.4	16.6 ± 3.6	**18.7* ± 2.1**	16.5 ± 2.1
Week 104		26.0 ± 4.8	25.6 ± 7.3	25.4 ± 9.3	24.0 ± 2.6	16.7 ± 3.4	17.2 ± 4.3	17.6 ± 4.8	18.2 ± 5.7
Average		24.8	24.2	24.3	24.2	16.4	16.5	16.8	16.4

The group mean achieved doses are summarised below.

Table B.6.5.4-5 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	3000	104	115
3 (mid)	10000	354	393
4 (high)	30000	1127	1247

The results show a higher test material intake for females when compared to males for each dose level.

F. OPHTHALMOLOGIC EXAMINATIONS

No abnormalities were observed.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.

In the week 52 satellite group no statistical significant effect on haematological parameters was noted.
In the main group the increase of haematocrit and decrease of platelet count is considered incidental and partly due to very low (RBC parameters) or high (platelets) control values when compared to other time points. These changes were only observed in one sex and at one time point. Compared to the control haematocrit values were

108 %, 111 % and 131 % for males and 99 %, 84 % and 96 % for females in the 3000, 10000 and 30000 ppm dose groups. Platelet count was decreased by 35%. No changes of these parameters have been observed at the other time points.

For the clinical chemistry findings, the observed statistical effects showed no response relationship, were observed in only one sex and/or were not consistent between the different time points. Therefore, it was concluded that there was no treatment-related adverse effect.

Statistically significant changes in haematology and blood chemistry are displayed in the tables below.

Table B.6.5.4-6 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in haematology

Parameter	Sex	Males				Females			
	Group #	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
	Dose (ppm)	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
	No. of rats	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Week 52 (satellite group)									
Haematocrit [%]		41.2 ± 0.9 (9)	38.9 ± 6.8	38.6 ± 7.3	41.6 ± 1.7	38.2 ± 2.9	39.2 ± 1.3	38.9 ± 2.0	37.5 ± 3.2
Platelet count [10^3/mm^3]		1208 ± 142 (9)	1198 ± 214	1176 ± 218	1252 ± 118	922 ± 120	952 ± 67	928 ± 130	1087 ± 177
Week 104 (main group)									
Haematocrit [%]		26.6 ± 7.6	28.7 ± 5.7	29.6 ± 7.4	**34.9* ± 4.9**	31.4 ± 5.0	31.0 ± 6.3	26.5 ± 6.5	↓0.2 ± 6.9
Platelet count [103/mm3]		1770 ± 409	1617 ± 313	1564 ± 475	**1172** ± 208**	1099 ± 419	1158 ± 292	1261 ± 254	1148 ± 197

* Statistically significant ($p \leq 0.05$) ; * Statistically significant ($p \leq 0.01$); numbers in braces represent the animal numbers of the respective endpoint different from 10 animals per group

Table B.6.5.4-6 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
	Dose (ppm)	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
	No. of rats	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Week 26 (satellite group)									
Alkaline phosphatase (ALP) [U/L]		69 ± 15	66 ± 15	81 ± 21	86 ± 15	30 ± 7	36 ± 11	35 ± 15	↑37 ± 20
Creatinine (Creat) [mg/dL]		0.91 ± 0.04	0.93 ± 0.08	0.90 ± 0.08	0.88 ± 0.05	1.04 ± 0.08	0.99 ± 0.08	**0.95* ± 0.06**	**0.93** ± 0.06**
Blood urea nitrogen (BUN) [mg/dL]		12.9 ± 1.4	12.7 ± 1.0	13.1 ± 1.9	13.0 ± 1.3	15.5 ± 1.5	15.3 ± 1.7	14.8 ± 1.4	15.4 ± 2.0
Total protein (TP) [g/dL]		6.69 ± 0.18	6.61 ± 0.25	6.50 ± 0.26	6.60 ± 0.25	7.29 ± 0.35	7.00 ± 0.37	7.10 ± 0.27	6.90 ± 0.30
Albumin (Alb) [g/dL]		3.05 ± 0.25	3.04 ± 0.06	3.04 ± 0.13	3.14 ± 0.16	4.06 ± 0.28	**3.73* ± 0.32**	4.02 ± 0.21	3.84 ± 0.24
Globulin (Glob) [g/dL]		3.65 ± 0.25	3.57 ± 0.25	3.46 ± 0.19	3.46 ± 0.16	3.23 ± 0.17	3.28 ± 0.18	3.08 ± 0.13	**3.06* ± 0.12**
Albumin/globulin ratio (A/G ratio)		0.84 ± 0.10	0.86 ± 0.07	0.88 ± 0.05	0.91 ± 0.05	1.26 ± 0.10	**1.14* ± 0.11**	1.30 ± 0.08	1.25 ± 0.08

Table B.6.5.4-6 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Glucose (Glue) [mg/dL]		136 ± 16	137 ± 7	145 ± 15	132 ± 9	137 ± 8	143 ± 22	136 ± 22	**119** ± 8
Total bilirubin (T.Bil) [mg/dL]		0.16 ± 0.02	0.16 ± 0.03	0.15 ± 0.01	0.17 ± 0.02	0.25 ± 0.05	0.20** ± 0.02	0.24 ± 0.02	0.22 ± 0.02
Week 52 (satellite group)									
Alkaline phosphatase (ALP) [U/L]		58 ± 11 (9)	75 ± 41	84 ± 54	79 ± 15	22 ± 8	28 ± 9	26 ± 10	**47** ± 25
Creatinine (Creat) [mg/dL]		1.01 ± 0.10 (9)	1.09 ± 0.11	1.08 ± 0.17	1.03 ± 0.09	0.98 ± 0.08	0.99 ± 0.09	0.92 ± 0.07	0.91 ± 0.07
Total protein (TP) [g/dL]		6.79 ± 0.35 (9)	6.76 ± 0.49	6.76 ± 0.31	6.75 ± 0.25	7.50 ± 0.30	7.55 ± 0.22	**7.19* ± 0.32**	7.20 ± 0.27
Albumin (Alb) [g/dL]		2.64 ± 0.10 (9)	2.50 ± 0.33	2.56 ± 0.45	2.61 ± 0.17	3.70 ± 0.25	3.73 ± 0.23	3.65 ± 0.21	3.45 ± 0.35
Globulin (Glob) [g/dL]		4.15 ± 0.31 (9)	4.26 ± 0.79	4.20 ± 0.66	4.15 ± 0.24	3.80 ± 0.22	3.82 ± 0.11	**3.54* ± 0.19**	3.75 ± 0.52
Albumin/globulin ratio (A/G ratio)		0.64 ± 0.05 (9)	0.61 ± 0.14	0.63 ± 0.15	0.63 ± 0.07	0.98 ± 0.09	0.98 ± 1.03	1.03 ± 0.07	0.94 ± 0.17
Glucose (Glue) [mg/dL]		143 ± 11 (9)	143 ± 17	138 ± 20	143 ± 32	143 ± 21	136 ± 15	136 ± 16	130 ± 15
Total bilirubin (T.Bil) [mg/dL]		0.20 ± 0.02 (9)	0.24 ± 0.09	0.24 ± 0.05	0.21 ± 0.02	0.29 ± 0.07	0.35 ± 0.11	0.33 ± 0.13	0.25 ± 0.06
Week 78 (satellite group)									
Alkaline phosphatase (ALP) [U/L]		48 ± 16	**89* ± 19**	74 ± 19	82 ± 29	32 ± 12 (8)	97 ± 147 (9)	34 ± 14 (8)	37 ± 21 (8)
Creatinine (Creat) [mg/dL]		1.03± 0.09	1.16 ± 0.22	1.01 ± 0.11	1.04 ± 0.21	1.05 ± 0.08 (8)	0.99 ± 0.06 (9)	1.01 ± 0.08 (8)	1.08 ± 0.22 (8)
Total protein (TP) [g/dL]		6.50 ± 0.32	6.14 ± 0.50	6.27 ± 0.37	6.53 ± 0.18	6.83 ± 0.38 (8)	6.69 ± 0.49 (9)	7.09 ± 0.44 (8)	7.08 ± 0.41 (8)
Albumin (Alb) [g/dL]		2.47 ± 0.49	1.95 ± 0.56	2.42 ± 0.31	2.68 ± 0.10	3.13 ± 0.42 (8)	3.72 ± 0.67 (9)	3.35 ± 0.32 (8)	2.84 ± 0.63 (8)
Globulin (Glob) [g/dL]		4.03 ± 0.33	4.19 ± 0.18	3.85 ± 0.43	3.85 ± 0.18	3.70 ± 0.35 (8)	3.97 ± 0.52 (9)	3.73 ± 0.30 (8)	4.25 ± 0.70 (8)
Albumin/globulin ratio (A/G ratio)		0.62 ± 0.17	0.47 ± 0.14	0.64 ± 0.14	0.70 ± 0.05	0.86 ± 0.17 (8)	0.71 ± 0.22 (9)	0.90 ± 0.10 (8)	0.70 ± 0.22 (8)
Glucose (Glue) [mg/dL]		131 ± 21	123 ± 18	140 ± 14	132 ± 13	126 ± 8 (8)	116 ± 21 (9)	116 ± 24 (8)	102 ± 24 (8)
Total bilirubin (T.Bil) [mg/dL]		0.22 ± 0.07	0.17 ± 0.05	0.20 ± 0.02	0.20 ± 0.03	0.25 ± 0.05 (8)	0.21 ± 0.05 (9)	0.23 ± 0.04 (8)	0.23 ± 0.04 (8)
Week 104 (main group)									
Alkaline phosphatase (ALP) [U/L]		64 ± 32	53 ± 25	84 ± 33	95 ± 85	39 ± 27	41 ± 30	61 ± 22	61 ± 26
Creatinine (Creat) [mg/dL]		1.11 ± 0.19	1.33 ± 0.33	1.19 ± 0.24	1.11 ± 0.38	1.14 ± 0.33	1.04 ± 0.09	1.03 ± 0.07	1.01 ± 0.07
Total protein (TP) [g/dL]		6.32 ± 0.38	6.45 ± 0.61	6.27 ± 0.68	6.49 ± 0.29	7.15 ± 0.28	6.90 ± 0.61	6.78 ± 0.35	7.02 ± 0.44
Albumin (Alb) [g/dL]		1.84 ± 0.37	1.88 ± 0.43	1.72 ± 0.30	2.24 ± 0.40	2.84 ± 0.40	2.61 ± 0.49	2.42 ± 0.41	2.71 ± 0.52

Table B.6.5.4-6 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Statistically significant changes in blood chemistry

Parameter	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Globulin (Glob) [g/dL]		4.48 ± 0.37	4.57 ± 0.51	4.55 ± 0.55	4.26 ± 0.35	4.31 ± 0.49	4.29 ± 0.51	4.37 ± 0.32	4.31 ± 0.56
Albumin/globulin ratio (A/G ratio)		0.42 ± 0.10	0.42 ± 0.11	0.38 ± 0.08	**0.53* ± 0.13**	0.67 ± 0.16	0.62 ± 0.14	0.56 ± 0.12	0.65 ± 0.18
Glucose (Glue) [mg/dL]		108 ± 20	92 ± 20	99 ± 16	115 ± 8	106 ± 12	112 ± 17	107 ± 17	115 ± 17
Total bilirubin (T.Bil) [mg/dL]		0.17 ± 0.02	0.23 ± 0.19	0.17 ± 0.04	0.18 ± 0.02	0.20 ± 0.05	0.20 ± 0.06	0.18 ± 0.08	0.19 ± 0.10

* Statistically significant ($p \leq 0.05$) ; * Statistically significant ($p \leq 0.01$); numbers in brackets represent the animal numbers of the respective endpoint different from 10 animals per group

G. URINALYSIS

Urinalysis showed a decrease in urinary pH at the high dose males and females. In addition, protein content appeared to be decreased. Urine also appeared to slightly darker. Metabolism of glyphosate after absorption from the intestine is minimal. Thus, most of the glyphosate is excreted *via* urine as the unchanged parent compound. In the urine glyphosate dissociates into the free acid, which can lead to a reduction of the urinary pH. Therefore, the reduced urinary pH might be of no toxicological significance.

Statistically significant changes in urinalysis parameters are displayed in **Table** .

Table B.6.5.4-7 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
pH									
Week 26 (satellite group)	5.0	-	-	-	-	-	-	-	-
	6.0	-	1	4	**10****	5	3	5	8
	6.5	5	3	5	-	3	2	2	2
	7.0	1	1	**1***	-	1	1	2	-
	7.5	1	2	-	-	-	3	1	-
	8.0	3	2	-	-	1	1	-	-
	8.5	-	1	-	-	-	-	-	-
Week 52 (satellite group)	5.0	-	-	-	4	-	-	-	5
	6.0	1	-	3	**6****	4	6	7	**5****
	6.5	2	5	4	-	1	3	2	-
	7.0	1	1	**3***	-	3	1	1	-
	7.5	4	4	-	-	2	-	-	-
	8.0	2	-	-	-	-	-	-	-
	8.5	-	-	-	-	-	-	-	-
	5.0	-	-	-	-	-	-	-	2 (8)
	6.0	2	-	4	**8***	6 (8)	9 (9)	6 (8)	6 (8)
	6.5	1	3	5	-	1 (8)	-	2 (8)	-

Table B.6.5.4-7 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
	Dose (ppm)	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
	No. of rats	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Week 78 (satellite group)	7.0	3	-	1	-	1 (8)	-	-	-
	7.5	1	2	-	-	-	-	-	-
	8.0	-	-	-	-	-	-	-	-
	8.5	-	-	-	-	-	-	-	-
Week 104 (main group)	5.0	-	-	-	1	-	-	-	3
	6.0	1	1	5	**9****	7	4	6	7
	6.5	4	6	1	-	1	4	4	-
	7.0	4	3	4	-	1	1	-	-
	7.5	1	-	-	-	1	1	-	-
	8.0	-	-	-	-	-	-	-	-
	8.5	-	-	-	-	-	-	-	-
Protein									
Week 26 (satellite group)	Negative	-	-	-	-	1	5	2	6
	Slight	1	3	3	4	7	2	4	3
	Moderate	5	5	7	**6***	1	2	3	1
	Severe	4	2	-	-	1	1	1	-
	Extreme	-	-	-	-	-	-	-	-
Week 52 (satellite group)	Negative	-	-	-	-	-	-	2	1
	Slight	-	-	-	1	4	5	2	5
	Moderate	5	5	7	7	3	1	5	3
	Severe	5	5	3	2	3	4	1	1
	Extreme	-	-	-	-	-	-	-	-
Week 78 (satellite group)	Negative	-	-	-	-	-	1 (9)	4 (8)	-
	Slight	-	-	-	-	5 (8)	4 (9)	1 (8)	1 (8)
	Moderate	-	-	1	-	1 (8)	3 (9)	3 (8)	2 (8)
	Severe	3	2	4	5	2 (8)	1 (8)	-	3 (8)
	Extreme	4	3	5	3	-	-	-	2 (8)
Week 104 (main group)	Negative	-	-	-	-	-	-	-	-
	Slight	-	-	-	-	1	-	3	5
	Moderate	-	-	-	-	4	6	2	2
	Severe	7	8	7	10	4	2	2	2
	Extreme	3	2	3	-	1	2	3	1
Appearance									
Week 26 (satellite group)	Colourless	-	-	-	-	-	-	-	-
	Pale yellow	-	-	-	-	1	-	-	-
	Yellow	10	8	10	10	9	10	10	9
	Yellow brown	-	2	-	-	-	-	-	1
	Brown	-	-	-	-	-	-	-	-
Week 52 (satellite group)	Colourless	-	-	-	-	-	-	-	-
	Pale yellow	1	-	-	-	-	-	-	-
	Yellow	9	9	9	10	10	10	10	10
	Yellow brown	-	1	1	-	-	-	-	-
	Brown	-	-	-	-	-	-	-	-

Table B.6.5.4-7 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Summary of urinalysis (satellite group)

Time point	Sex	Males				Females			
	Group #	G1	G2	G3	G4	G1	G2	G3	G4
	Dose (ppm)	0	3000	10000	30000	0	3000	10000	30000
	No. of rats	10	10	10	10	10	10	10	10
Week 78 (satellite group)	Colourless	-	-	-	-	-	-	-	-
	Pale yellow	2	1	-	-	1 (8)	4 (9)	3 (8)	-
	Yellow	5	3	10	8	7 (8)	5 (9)	5 (8)	8 (8)
	Yellow brown	-	-	-	-	-	-	-	-
	Brown	-	1	-	-	-	-	-	-
Week 104 (main group)	Colourless	-	-	-	-	1	1	-	-
	Pale yellow	-	3	-	1	7	1	3	2
	Yellow	6	6	6	7	2	7	6	**8***
	Yellow brown	3	1	2	1	-	-	-	-
	Brown	1	-	2	1	-	**1***	**1***	-
Urine volume [mL]									
Week 26 (satellite group)		10.2 ± 2.4	↑12.5 ± 4.9	↑13.3 ± 5.1	↑12.3 ± 6.2	13.5 ± 5.9	↑15.8 ± 7.9	↓12.4 ± 5.1	↓11.7 ± 6.3
Week 52 (satellite group)		7.0 ± 3.5	↑10.8 ± 5.3	↑11.6 ± 12.2	↑9.2 ± 3.2	15.8 ± 5.0	↑25.2 ± 13.2	↓13.9 ± 9.1	↓11.3 ± 7.0
Week 78 (satellite group)		9.0 ± 12.5	↑20.9 ± 30.2	↓7.2 ± 4.1	↑11.6 ± 13.3	12.1 ± 6.9 (8)	↑19.4 ± 9.7 (9)	↑16.7 ± 11.8 (8)	↓7.8 ± 3.0 (8)
Week 104 (main group)		11.4 ± 7.0	**↑29.0 ± 16.6***	↑17.2 ± 13.4	↓10.4 ± 4.8	18.8 ± 9.3	↑27.6 ± 31.7	↑19.4 ± 10.0	↓18.5 ± 8.3

* Statistically significant ($p \leq 0.05$) ; * Statistically significant ($p \leq 0.01$); numbers in braces represent the animal numbers of the respective endpoint different from 10 animals per group

H. NECROPSY

In the high dose group significant increases in incidence of distension of the caecum were observed in both sexes, accompanied by soiled fur in the perianal region in males. Moreover, significant increases in absolute and relative weights of the caecum in both sexes in the high and mid dose group were seen, but not associated with histopathological abnormalities (Tables below).

The only observed changes in organ weight were an increase in relative brain (+12%), testes (+14.5%) and liver (+12%) weight in high dose males at 26 week and an increase in relative kidney weight (+15%) in high dose females at 26 weeks. Considering that the effects are only slight, not observed at other time point and without concomitant histopathological finding the increase in relative brain, testes and liver weight were not considered as adverse.

Table B.6.5.4-8 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (males)

mg/kg bw/d		Group mean values in male rats (Satellite)							
		No. of animals	Body Weight (g)	Brain (mg)	Liver (g)	Kidneys (mg)	Adrenals (mg)	Testes (mg)	Cecum (mg)
Week 26	0	10	660 ± 45	2224 ± 91	15.32 ± 2.08	3335 ± 362	60.8 ± 8.4	3593 ± 222	3310 ± 653

Table B.6.5.4-8 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (males)

		Group mean values in male rats (Satellite)							
mg/kg bw/d		**No. of animals**	**Body Weight (g)**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Testes (mg)**	**Cecum (mg)**
(satellite group)	3000	10	647 ± 54	2223 ± 47	14.88 ± 1.91	3453 ± 252	65.1 ± 9.0	3525 ± 200	3405 ± 956
	10000	10	652 ± 71	2268 ± 121	15.46 ± 2.01	3351 ± 347	61.2 ± 4.4	3648 ± 219	**4500* ± 1227 (+36%)**
	30000	10	594 ± 51	2257 ± 41	15.49 ± 2.04	3305 ± 356	57.0 ± 7.5	3684 ± 191	**6234** ± 1159 +88%)**
Week 52 (satellite group)	0	10	782 ± 58	2375 ± 103	18.62 ± 2.38	3936 ± 588	60.4 ± 9.6	3697 ± 189	3691 ± 529
	3000	10	798 ± 63	2339 ± 58	23.05 ± 15.28	4461 ± 1824	63.6 ± 9.4	3671 ± 309	3272 ± 1125
	10000	10	791 ± 130	2359 ± 107	20.62 ± 8.37	4528 ± 2891	65.1 ± 12.0	3627 ± 389	4493 ± 1386
	30000	10	701 ± 74	2296 ± 90	18.16 ± 2.64	3936 ± 477	59.1 ± 9.2	3595 ± 490	**5896** ± 823 (+60%)**
Week 78 (satellite group)	0	6	851 ± 94	2376 ± 63	20.42 ± 4.38	4722 ± 1273	79.6 ± 21.9	3317 ± 885	3719 ± 666
	3000	5	784 ± 119	2475 ± 151	17.83 ± 1.75	4491 ± 584	120.0 ± 95.7	3343 ± 784	4427 ± 978
	10000	10	849 ± 75	2417 ± 56	21.35 ± 3.71	4542 ± 731	92.7 ± 40.9	3313 ± 499	4807 ± 975
	30000	8	738 ± 90	2341 ± 55	21.36 ± 5.00	4516 ± 1135	73.3 ± 26.3	3686 ± 385	**6873** ± 1826 (+85%)**
Week 104 (main group)	0	10	773 ± 121	2400 ± 67	18.44 ± 2.40	4588 ± 686	107.0 ± 44.0	3720 ± 1059	3413 ± 909
	3000	10	746 ± 67	2371 ± 134	19.23 ± 3.11	5656 ± 1513	110.5 ± 31.5	3040 ± 809	3799 ± 1990
	10000	10	768 ± 196	2337 ± 126	18.67 ± 3.15	4808 ± 1275	92.4 ± 18.7	3362 ± 446	4539 ± 961 (+32%)
	30000	10	739 ± 115	2432 ± 117	17.70 ± 2.24	4267 ± 1039	85.3 ± 19.9	3564 ± 465	**7272** ± 2841 (+113%)**

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table B.6.5.4-9 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (males)

		Group mean values in male rats (Satellite)						
mg/kg bw/d		**No. of animals**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Testes (mg)**	**Cecum (mg)**
Week 26 (satellite group)	0	10	0.34 ± 0.02	2.32 ± 0.18	0.51 ± 0.04	0.009 ± 0.001	0.55 ± 0.06	0.50 ± 0.08
	3000	10	0.35 ± 0.03	2.30 ± 0.17	0.54 ± 0.05	0.010 ± 0.001	0.55 ± 0.07	0.53 ± 0.16
	10000	10	0.35 ± 0.04	2.37 ± 0.11	0.52 ± 0.05	0.009 ± 0.001	0.57 ± 0.08	0.70 ± 0.23

Table B.6.5.4-9 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (males)

		Group mean values in male rats (Satellite)						
mg/kg bw/d		**No. of animals**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Testes (mg)**	**Cecum (mg)**
	30000	10	**0.38** ± 0.04**	**2.60** ± 0.18**	0.56 ± 0.04	0.010 ± 0.001	**0.63* ± 0.07**	**1.06** ± 0.25**
Week 52 (satellite group)	0	10	0.31 ± 0.02	2.38 ± 0.25	0.50 ± 0.06	0.008 ± 0.001	0.47 ± 0.04	0.47 ± 0.07
	3000	10	0.30 ± 0.03	3.02 ± 2.46	0.58 ± 0.31	0.008 ± 0.002	0.46 ± 0.05	0.42 ± 0.16
	10000	10	0.31 ± 0.04	2.69 ± 1.41	0.61 ± 0.48	0.008 ± 0.002	0.47 ± 0.08	0.58 ± 0.20
	30000	10	0.33 ± 0.04	2.59 ± 0.25	0.57 ± 0.08	0.008 ± 0.001	0.52 ± 0.09	**0.85** ± 0.13**
Week 78 (satellite group)	0	6	0.28 ± 0.03	2.39 ± 0.33	0.55 ± 0.13	0.009 ± 0.002	0.39 ± 0.11	0.45 ± 0.11
	3000	5	0.32 ± 0.07	2.32 ± 0.45	0.59 ± 0.14	0.017 ± 0.017	0.42 ± 0.05	0.58 ± 0.17
	10000	10	0.29 ± 0.03	2.52 ± 0.44	0.54 ± 0.08	0.011 ± 0.005	0.39 ± 0.07	0.57 ± 0.15
	30000	8	0.32 ± 0.05	2.99 ± 1.16	0.64 ± 0.28	0.011 ± 0.006	0.51 ± 0.11	**0.97** ± 0.40**
Week 104 (main group)	0	10	0.32 ± 0.05	2.42 ± 0.36	0.61 ± 0.15	0.014 ± 0.008	0.48 ± 0.11	0.45 ± 0.15
	3000	10	0.32 ± 0.03	2.59 ± 0.42	0.77 ± 0.22	0.015 ± 0.006	0.41 ± 0.10	0.51 ± 0.24
	10000	10	0.32 ± 0.07	2.52 ± 0.25	0.67 ± 0.25	0.013 ± 0.004	0.45 ± 0.09	0.62 ± 0.17
	30000	10	0.34 ± 0.05	2.43 ± 0.38	0.59 ± 0.17	0.012 ± 0.003	0.49 ± 0.10	**0.97** ± 0.29**
*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.								

Table B.6.5.4-10 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)						
mg/kg bw/d		**No. of animals**	**Body Weight (g)**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Cecum (mg)**
Week 26 (satellite group)	0	10	361 ± 39	2058 ± 74	8.28 ± 0.87	1912 ± 155	72.3 ± 10.6	2747 ± 596
	3000	10	340 ± 49	2044 ± 54	8.04 ± 1.20	1936 ± 215	67.7 ± 8.9	2773 ± 716
	10000	10	356 ± 356	2022 ± 38	8.36 ± 0.92	1947 ± 158	69.6 ± 10.2	3055 ± 1071
	30000	10	321 ± 37	2040 ± 91	8.05 ± 1.24	1929 ± 131	66.2 ± 8.6	**5359** ± 1572** +95%)
Week 52 (satellite group)	0	10	419 ± 86	2061 ± 67	10.05 ± 1.90	2110 ± 193	72.0 ± 15.5	2113 ± 683
	3000	10	484 ± 88	2077 ± 109	10.75 ± 1.74	2307 ± 312	89.0 ± 22.6	2580 ± 449
	10000	10	479 ± 479	2076 ± 101	10.50 ± 1.46	2303 ± 140	77.6 ± 12.5	**2995* ± 595 (+42%)**

Table B.6.5.4-10 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Absolut organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)						
mg/kg bw/d		**No. of animals**	**Body Weight (g)**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Cecum (mg)**
	30000	10	402 ± 39	2088 ± 69	10.45 ± 2.34	2602 ± 1138	73.1 ± 11.8	**4806** ± 772 (+127%)**
Week 78 (satellite group)	0	8	555 ± 101	2131 ± 77	12.78 ± 2.05	2466 ± 278	91.2 ± 32.1	2911 ± 726
	3000	9	505 ± 88	2133 ± 144	12.23 ± 2.34	2732 ± 406	134.2 ± 88.4	3101 ± 3101
	10000	8	481 ± 65	2147 ± 78	10.98 ± 1.25	2512 ± 281	88.1 ± 32.9	3947 ± 3947
	30000	8	433 ± 146	2141 ± 87	11.01 ± 2.95	2612 ± 328	101.8 ± 34.9	4857 ± 4857
Week 104 (main group)	0	10	528 ± 92	2143 ± 119	14.18 ± 3.20	3224 ± 1112	117.3 ± 50.4	2670 ± 1021
	3000	10	544 ± 93	2147 ± 97	14.47 ± 4.19	2978 ± 351	101.4 ± 35.2	**4193* ± 1287**
	10000	10	567 ± 71	2160 ± 80	14.98 ± 2.60	3001 ± 236	154.1 ± 85.3	3741 ± 1536 (+40%)
	30000	10	540 ± 80	2128 ± 64	14.62 ± 1.99	2991 ± 348	114.0 ± 37.5	**4909** ± 1318** (+84%)

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table B.6.5.4-11 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)					
mg/kg bw/d		**No. of animals**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Cecum (mg)**
Week 26 (satellite group)	0	10	0.58 ± 0.06	2.30 ± 0.16	0.53 ± 0.05	0.020 ± 0.003	0.78 ± 0.23
	3000	10	0.61 ± 0.08	2.37 ± 0.24	0.58 ± 0.07	0.020 ± 0.004	0.83 ± 0.23
	10000	10	0.57 ± 0.06	2.35 ± 0.16	0.55 ± 0.04	0.020 ± 0.004	0.88 ± 0.34
	30000	10	0.64 ± 0.07	2.50 ± 0.19	**0.61* ± 0.06**	0.021 ± 0.003	**1.71** ± 0.59**
Week 52 (satellite group)	0	10	0.51 ± 0.09	2.42 ± 0.27	0.52 ± 0.08	0.018 ± 0.005	0.53 ± 0.20
	3000	10	0.44 ± 0.08	2.33 ± 0.14	0.48 ± 0.08	0.019 ± 0.004	0.56 ± 0.17
	10000	10	0.44 ± 0.07	2.21 ± 0.22	0.50 ± 0.09	0.017 ± 0.004	0.65 ± 0.21
	30000	10	0.52 ± 0.04	2.62 ± 0.69	0.66 ± 0.32	0.018 ± 0.004	**1.20** ± 0.15**
Week 78 (satellite group)	0	8	0.40 ± 0.08	2.32 ± 0.24	0.46 ± 0.08	0.017 ± 0.006	0.54 ± 0.17
	3000	9	0.43 ± 0.08	2.42 ± 0.16	0.55 ± 0.05	0.026 ± 0.017	0.63 ± 0.18
	10000	8	0.45 ± 0.06	2.30 ± 0.21	0.53 ± 0.08	0.018 ± 0.006	0.84 ± 0.24

Table B.6.5.4-11 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Relative organ weights at interim/terminal kills - Group mean values (females)

		Group mean values in female rats (Satellite)					
mg/kg bw/d		**No. of animals**	**Brain (mg)**	**Liver (g)**	**Kidneys (mg)**	**Adrenals (mg)**	**Cecum (mg)**
	30000	8	0.56 ± 0.22	2.61 ± 0.28	0.66 ± 0.21	0.027 ± 0.017	**1.22* ± 0.74**
Week 104 (main group)	0	10	0.42 ± 0.06	2.75 ± 0.70	0.65 ± 0.35	0.024 ± 0.014	0.52 ± 0.20
	3000	10	0.41 ± 0.07	2.68 ± 0.64	0.56 ± 0.09	0.019 ± 0.007	**0.80* ± 0.30**
	10000	10	0.39 ± 0.07	2.67 ± 0.50	0.54 ± 0.09	0.028 ± 0.015	0.66 ± 0.27
	30000	10	0.40 ± 0.06	2.72 ± 0.27	0.56 ± 0.09	0.022 ± 0.009	**0.91** ± 0.24**

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

The incidences of thickened areas in the skin of the tail, corresponding to the tail mass in the clinical observations, were increased in the mid and high dose group. The lesion was diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess. An increased incidence of hair loss was also observed in high-dosed females (Table and B.6.5.4-13).
Besides the observed effects on the cecum and skin a statistically significant decrease in myocardial atrophy/fibrosis was observed at high dose females which is not adverse and unlikely to be treatment related.

Table B.6.5.4-12 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Gross pathology

		Males				**Females**			
ppm		**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Findings/Number of animals (Satellite)									
Digestive system	Intestine								
	Distention of cecum								
	Week 26	0/10	0/10	0/10	**9/10****	0/10	0/10	2/10	**10/10****
External appearance	Fur								
	Soiled in perianal region								
	Week 52	0/10	0/10	0/10	**5/10***	0/10	0/10	0/10	2/10
Digestive system	Intestine								
	Distention of cecum								
	Week 78	0/6	0/5	0/10	**6/8****	0/8	0/9	0/8	**4/8***
Findings/Number of animals (Main group) Week 104 – Terminal Kill									
Digestive system	Intestine								
	Distention of cecum	0/18	1/20	1/18	**16/29****	-	-	-	-
Integumentary system	Skin								
	Callosity in paw	12/18	11/20	11/18	**8/29****	6/15	8/19	**13/16***	7/14
External Appearance	Fur								
	Loss of tactile hair	-	-	-	-	0/35	**5/31***	1/34	1/36
	Soiled in fore-limb	3/32	**11/30***	2/32	4/21	-	-	-	-
Urinary system	Kidney								
	Coarse surface	3/32	5/30	6/32	**7/21***	-	-	-	-
	Uterus								

Table B.6.5.4-12 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Gross pathology

		Males				Females			
ppm		**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Genital system	Mass(es)	-	-	-	-	4/15	**0/19***	1/16	2/14
Endocrine system	Pituitary								
	Mass(es)	19/32	16/30	**10/32***	13/21	-	-	-	-
	Adrenals								
	Enlargement	-	-	-	-	8/35	3/31	1/34	2/36
Integumentary system	Skin								
	Hair loss	-	-	-	-	3/35	5/31	5/34	**11/36***
	Callosity in paw	-	-	-	-	3/35	6/31	7/34	**10/36***
	Thickened area	4/32	3/30	1/32	**9/21***	0/35	0/31	4/34	**5/36***
Body cavities	Thoracic cavity								
	Hydrothorax	2/31	0/30	**8/32***	2/21	-	-	-	-

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table B.6.5.4-12 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic non-neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Total animals investigated	76	75	80	78	78	79	78	78
Cardiovascular System								
Heart								
NAD	24	18	22	27	53	52	53	65
Myocardial atrophy/fibrosis	47	49	45	45	22	19	20	**8****
Endocardial hyperplasia	0	1	1	0	0	0	0	1
Myocarditis	6	6	10	6	3	6	4	2
Endocardial mineralisation	0	0	1	4	1	0	0	1
Epicarditis	0	0	1	0	0	0	0	0
Auricular thrombus	0	1	2	1	0	0	2	0
Ventricular thrombus	0	0	1	0	0	0	0	0
Haemorrhage	0	0	1	0	0	0	0	0
Arteritis	0	2	0	1	0	0	0	0
Integumentary System								
Skin								
NAD	35	32	44	35	62	56	45	52
Epithelial hyperplasia	2	0	2	0	0	0	0	0
Necrosis	0	1	0	0	0	0	1	0
Dermatitis	3	4	4	3	2	1	3	2
Epidermal cyst	3	0	1	4	0	0	2	1
Erosion/ulcer	1	0	0	0	0	1	0	0
Fibrosis	1	1	0	2	0	0	0	0
Follicular epithelial hyperplasia	0	0	0	1	0	0	0	0
Folliculitis/follicular abscess	5	7	1	9	0	1	**6***	4
Follicular dilatation	3	5	4	3	0	0	0	0
Follicular hyperkeratosis	7 (9.2%)	5 (6.7%)	2 (2.5%)	**23** (29.5%)**	0 (0%)	2 (2.5%)	2 (2.6%)	**6* (7.7%)**
Plantar granuloma	27	25	21	19	10	17	**22***	17
Palmar granuloma	1	0	0	0	0	0	0	0

Table B.6.5.4-12 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic non-neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Subcutaneous cyst	0	0	1	0	0	0	0	0
Subcutaneous oedema	0	0	2	0	0	0	0	1
Subcutaneous inflammation	3	2	0	1	0	0	0	1
Subcutaneous abscess	2	1	1	2	0	2	1	2

Numbers in brackets represent the animal number of the respective finding
* Significantly different from control ($p \leq 0.05$ %)
** Significantly different from control ($p \leq 0.01$ %)
NAD= No abnormalities detected

All changes regarding neoplastic lesions were not statistically significant according to the study report. However, based on the RMS re-assessment, there appeared to be a slight non-significant increase in skin keratoacanthomas in male rats (7/78 compared to 4/76 in controls). Further, an increase in skin basal cell tumours was observed in male rats at the top dose. Upon statistical re-analysis of these findings by an external statistician, for the increase in skin keratoacanthomas no statistically significant trend was observed (P (two-sided) = 0.21 for the extended Mantel-Haenszel test (stratified Cochran-Armitage trend)). However, for the skin basal cell tumours, a statistically significant trend was observed (P (two-sided) = 0.001 for the extended Mantel-Haenszel test (stratified Cochran-Armitage trend)). This observation is further discussed in Volume 1.

Table B.6.5.4-13 HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats ([redacted] 1997): Histopathology of main and satellite groups – Incidence of microscopic neoplastic lesions

	Males				Female			
ppm	**0**	**3000**	**10000**	**30000**	**0**	**3000**	**10000**	**30000**
Total animals investigated	76	75	80	78	78	79	78	78
Integumentary System								
Skin								
Keratoacanthoma HCD (2 studies, years 1995-2000) Males: 2/50 (4%) and 4/50 (8%)	4	3	0	7	0	0	0	1
Basal cell tumours HCD (2 studies, years 1995-2000) Males: 0/50 (0%) for both studies	0	0	0	4#[a]	0	0	0	0

3 adenoma, 1 carcinoma; [a] P (two-sided) for trend = 0.001

Assessment and conclusion by applicant:
In conclusion, HR-001 was not carcinogenic in the Sprague-Dawley rats following continuous dietary exposure of up to 30000 ppm for 24 months. The NOAEL for toxicity is 3000 ppm, corresponding to 104.0 mg/kg bw/day for males and 114.7 mg/kg bw/day for females.

Assessment and conclusion by RMS:
Overall the assessment made by the applicant is agreed with. The NOAEL of 3000 ppm is agreed upon based on increased caecum weight at 10000 ppm.
The RMS has added that increased incidence in skin basal cell tumours and skin keratoacanthoma were observed at the top dose in males (Table B.6.5.4-13). The relevance of these findings are further discussed in Volume 1.

The NOAEL of 3000 ppm is in line with the previous assessment made in the RAR, 2015

B.6.5.5. Long-term toxicity – rat, study 5

Data point:	CA 5.5/005
Report author	[redacted]
Report year	1996
Report title	Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats
Report No	886.C.C-R
Document No	Not reported
Guidelines followed in study	OECD 453 (1981)
Deviations from current test guideline (OECD 453, 2018)	The following deviations were noted from OECD 453 : - individual animals exceed the 20 % range in initial body weight; - mortality was observed only once per day (instead of twice per day); - haematological parameters: prothrombin time, activated partial thromboplastin time, reticulocyte count were not observed; - clinical chemistry: P, Cl, Na, K, cholesterol, bilirubin, creatinine, were not observed; - urinalysis: osmolality/spec gravity and occult blood was not determined; - organ weights of epididymis, heart, spleen, thyroid/parathyroid and uterus were not determined. In addition, only 10 animals per dose were included in the organ weight measurement. - Histopathological examination of the Harderian gland, cervix, coagulating glands, lacrimal gland, rectum and vagina was not performed.
Previous evaluation	Yes, accepted in the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid, Category 2a **Conclusion AGG:** Some deviations were noted compared to the current OECD test guideline. This mainly consisted of missing parameters which were not included in the haematological, clinical chemistry and urinalysis assessment. In addition, the organ weights of only 10 animals per dose were assessed instead of all animals as is required by OECD 453. Based on these limitation the study is concluded to be acceptable but with restrictions (reliable with restrictions).

Full summary

The chronic toxicity and carcinogenic potential of glyphosate technical was assessed in a 24-month feeding study in male and female Wistar rats. Groups of 50 rats per sex received daily dietary doses of 0, 100, 1000, and 10000 ppm glyphosate technical (equal to 0, 6.3, 59.4 and 595.2 mg/kg bw/day in males and 0, 8.6, 88.5 and 886.0 mg/kg bw/day in females). In addition, one vehicle control 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. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis, as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on body weight gain or food consumption. The only treatment-related significant changes observed in haematological, biochemical parameters was an increase in ALP in high dose female.

Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related and dose-dependent effects except for an increase in cataract in high dose males.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White odourless crystals
Lot/Batch #:	60; 046
Purity:	96.8%; 96%
Stability of test compound:	More than two years at ambient temperature

2. Vehicle and/or positive control:

Diet

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 90 – 179 g, females: 80 – 151 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" (M/S Lipton India Ltd, India), *ad libitum*
Water:	Deep bore well water treated with charcoal filter and UV rays, *ad libitum*
Housing:	Initially in groups of five per sex in polypropylene cages and in groups of three from Week 12 onwards.
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 40 - 70 % Air changes: not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1992-03-04 to 1994-03-04

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Wistar rats per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 740.6 mg/kg bw/day for 24 months respectively) glyphosate technical. In additional one vehicle control 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.

Test diets were prepared fortnightly by mixing a known amount of the test substance with a small amount of basal diet. This pre-mix was then added to larger amount of basal diet and blended for further 20 minutes.

The stability of the test substance in food was determined at day 1, day 15 and day 30 in an in-house stability study at 2000 and 20000 ppm. It was stated that homogeneity of samples was also determined but no results were provided.

Observations
Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Rats were examined for toxic signs and pre-terminal deaths once a day. Ophthalmic examination was done at the start of the study and at termination.

Body weight
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 and compound intake
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.

Haematology and clinical chemistry

Haematology
Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. The following parameters were measured: Haemoglobin, haematocrit, erythrocyte count, clotting time and total leukocyte count and differential leukocyte count.

Blood chemistry
At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. The following parameters were measured: Total proteins, albumin, ALT, AST, GGT, ALP, blood urea nitrogen and blood glucose.

Urinalysis
Individual urine samples were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. The following measurements were made: Volume, appearance, pH, nitrite, urobilinogen, bilirubin, erythrocytes, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology
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.

The following organ weights were determined from 10 rats per sex per group: adrenals, brain, gonads, kidneys and liver.

Tissue samples were taken from the following organs: adrenals, aorta (main group animals), bone & bone marrow (sternum and femur incl. joint), brain, caecum, colon, duodenum, epididymides (main group animals), eyes (with optic nerve), heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric, mandibular and mediastinal), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, tumour/mass, urinary bladder and uterus.

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.

Statistics
Using specific computer programs, body weight, net body weight gain, food consumption, haematology, clinical chemistry and organ weight data of different groups were compared by Bartlett's test for homogeneity of intra group variances. When the variances proved to be heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intra group variances were subjected to one-way analysis of variance

(ANOVA - Snedecor and Cochran, 1980). When 'F' value was significant, Dunnett's pair wise comparison (Scheffe, 1953) of means of treated groups with control mean
was done individually.

Net food intake (g/kg bw/day) and test compound intake (mg/kg bw/day) was calculated for the whole study period using calculated means and food intake was statistically analysed by the procedure given above. Incidence of gross, histopathological changes of mass(es) and incidence of benign and malignant neoplasia in the treatment groups were statistically compared with control group by Z-test wherever it was applicable/necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto's incidental tumour analysis.
When a significant difference to the control was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

Analyses for achieved concentrations showed that the diet preparations of the control, low, mid- and high dose group were within an acceptable range. The mean achieved concentrations of the test substance of eight batches of the prepared test substance diets were 0.0, 99.1 ± 4.7, 995.3 ± 36.8 and 9993.1 ± 277.5 ppm, for the control, low, mid and high dose group, respectively.

B. MORTALITY

There were no treatment-related deaths observed during the study.
The numbers of pre-terminal deaths in the carcinogenicity study groups are displayed in the table below.

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical signs of toxicity observed during the study.

Table B.6.5.5-1 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of toxic signs, physical examination and pre-terminal deaths

Conditions	Sex	Males						Females					
	Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
	Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
	Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
	No. of rats	10	20	50	50	50	50	10	20	50	50	50	50
Dull/Lethargy		0	0	0	0	0	0	0	0	0	0	0	0
Weak/Loss of body weight/Emaciation		0	0	13	20	21	17	0	2	10	16	7	14
Nasal discharge		1	5	42	46	41	40	2	3	33	33	34	36
Snuffling/Respiratory problems		3	7	40	42	42	40	1	1	28	32	37	37
Epistaxis		0	0	0	0	0	0	0	0	0	0	0	0
Conjunctivitis/Lacrimation		0	0	1	4	0	0	0	0	0	1	0	1
Microphthalmia		0	0	2	1	0	5	0	1	1	2	2	2
Exophthalmia/Swelling of Eye		0	0	2	0	0	2	0	0	0	0	0	1
Blindness/Opacity		1	0	9	1	2	6	0	0	2	1	4	1
Cataract		0	0	6	5	1	7	0	0	2	1	3	2
Ear infection		0	0	0	0	0	0	0	0	0	0	0	0
Circling symptom		0	0	4	5	6	4	1	0	9	9	8	2
Soft stool/Diarrhoea		3	3	16	7	15	3	0	0	1	0	0	0

Table B.6.5.5-1 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of toxic signs, physical examination and pre-terminal deaths

Conditions	Sex	Males						Females					
	Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
	Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
	Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
	No. of rats	10	20	50	50	50	50	10	20	50	50	50	50
Obesity/Distended abdomen		0	0	3	1	2	1	2	3	5	7	5	3
Urine incontinence		0	0	5	3	2	1	0	0	1	12	3	1
External Genitalia affection		0	0	3	2	2	2	0	0	0	0	0	0
Piloerection/Rough hair coat		0	0	0	0	0	1	0	0	0	0	0	0
Alopecia		0	0	18	19	18	17	0	0	7	5	5	4
Hyperesthesia		0	0	0	0	0	0	0	0	0	0	0	0
Paralysis		0	0	0	0	0	0	0	0	0	0	0	0
Mass/Growth		0	0	6	8	6	2	0	0	10	9	3	7
Injury/Wound		0	0	0	3	1	1	0	0	3	2	0	3
Overgrown tooth		0	0	0	0	0	0	0	0	0	1	0	0
Moribund Sacrifice		0	0	3	8	9	5	0	0	10	10	7	11
Moribund Sacrifice after 730 days		0	0	0	0	0	0	0	0	0	2	0	0
Mortality		0	0	23	21	23	16	0	0	16	11	10	18
Mortality after 730 days		0	0	4	1	0	0	0	0	0	1	0	0
Survival		0	0	20	20	18	29	0	0	24	26	33	21

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study. During the first weeks of exposure statistically significant decreases of body weights were observed in males only. As the slight reduction of body weight (<10 %) was only observed in males at the beginning of the study and appeared to be related to a reduction in the initial body weight it was not considered to be adverse.

Table B.6.5.5-2 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Body weights

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Initial	142 ± 19.7	148 ± 16.1	139 ± 14.0	133 ± 17.3	139 ± 17.4	**128* ± 16.1**	123 ± 11.9	114 ± 14.4	116 ± 12.2	113 ± 13.3	112 ± 11.7	111 ± 14.2
Week 1	183 ± 23.8	176 ± 19.6	171 ± 19.0	**158* ± 22.3**	171 ± 22.9	**161*± 20.6**	141 ± 12.0	135 ± 16.7	132 ± 12.8	134 ± 13.9	130 ± 12.5	129 ± 13.5
Week 2	215 ± 21.1	203 ± 20.3	198 ± 19.2	191 ± 23.1	192 ± 26.2	**186* ± 24.5**	151 ± 11.7	149 ± 16.5	144 ± 14.8	146 ± 14.1	146 ± 12.2	140 ± 16.4

Table B.6.5.5-2 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Body weights

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Week 3	244 ± 27.2	234 ± 22.1	228 ± 21.0	**216* ± 26.0**	**215* ± 25.2**	**215* ± 28.4**	171 ± 15.3	161 ± 16.8	159 ± 13.9	160 ± 15.5	158 ± 14.1	154 ± 17.0
Week 4	267 ± 22.2	257 ± 22.1	248 ± 23.6	239 ± 26.8	**236* ± 27.6**	**236* ± 26.7**	185 ± 15.9	176 ± 16.3	172 ± 15.1	175 ± 16.9	173 ± 15.4	167 ± 17.3
Week 5	279 ± 17.1	273 ± 23.4	261 ± 21.2	255 ± 24.8	258 ± 30.4	253 ± 29.2	190 ± 13.8	182 ± 14.4	178 ± 15.7	**184* ± 17.0**	181 ± 16.7	175 ± 17.2
Week 6	283 ± 13.7	283 ± 24.1	274 ± 21.7	266 ± 24.8	270 ± 29.9	266 ± 29.1	194 ± 12.2	180 ± 11.8	186 ± 15.4	191 ± 16.4	184 ± 18.3	181 ± 19.5
Month 12	408 ± 21.3	410 ± 35.2	398 ± 30.2	**384* ± 37.8**	401 ± 41.7	390 ± 37.5	261 ± 62.7	253 ± 38.0	241 ± 26.1	**254* ± 28.1**	246 ± 32.3	248 ± 32.4
Month 24	-	-	401 ± 48.3	386 ± 45.7	407 ± 72.1	396 ± 41.6	-	-	278 ± 25.9	289 ± 33.5	282 ± 26.2	279 ± 39.7

* Statistically significant ($p \leq 0.05$)

Table B.6.5.5-3 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Body weight gain

Sex	Males						Females					
Period	1 to 12 month		1 to 24 month				1 to 12 month		1 to 24 month			
Group #	G1IS	G4IS	G1	G2	G3	G4	G1IS	G4IS	G1	G2	G3	G4
Dose (ppm)	0	10000	0	100	1000	10000	0	10000	0	100	1000	10000
Week 13	190 ± 19.4	186 ± 23.8	187 ± 22.9	185 ± 22.4	189 ± 26.3	194 ± 23.6	93 ± 16.4	96 ± 20.1	94 ± 16.0	101 ± 14.7	100 ± 22.5	96 ± 18.0
Month 6	239 ± 22.6	237 ± 30.0	234 ± 26.6	233 ± 32.6	236 ± 33.8	240 ± 32.5	108 ± 25.7	115 ± 27.4	108 ± 20.3	117 ± 17.4	116 ± 24.0	115 ± 21.7
Month 12	266 ± 22.1	262 ± 31.6	259 ± 27.9	251 ± 37.3	262 ± 36.8	262 ± 32.5	138 ± 58.4	139 ± 42.5	126 ± 27.0	**141* ± 23.7**	134 ± 30.7	137 ± 29.4
Month 18	-	-	270 ± 31.7	261 ± 49.0	260 ± 39.4	273 ± 40.1	-	-	154 ± 31.0	169 ± 37.8	162 ± 32.1	162 ± 36.2
Month 24	-	-	260 ± 48.4	253 ± 43.5	269 ± 67.9	265 ± 43.8	-	-	162 ± 25.4	176 ± 33.1	171 ± 29.5	162 ± 36.2

* Statistically significant ($p \leq 0.05$)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

Average food intake per day were 60.4, 62.7, 59.4 and 59.5 g/kg bw/day for the control, low, mid and high dose males, respectively and 93.1, 85.9, 88.5 and 88.6 g/kg bw/day for females of the control, low, mid and high dose groups, respectively.

The group mean achieved doses are summarised below.

Table B.6.5.5-4 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Group mean achieved dose levels in the main groups

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	100	6.3	8.6
3 (mid)	1000	59.4	88.5
4 (high)	10000	595.2	886.0

The results show a higher test material intake for females when compared to males for each dose level.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

No effect on haematological parameters were observed. Significant changes either showed no dose-response relationship or were observed on a single time point only.

The following significant dose related changes of the blood chemistry parameters were seen at the high dose:

- decrease in GGT activity at 12 months in male rats
- decrease in Albumin level at 6 months in female rats
- decrease in glucose at 12, 18 and 24 months in male rats although a dose response relationship was not always observed.
- increase in ALP (alkaline phosphatase) activity at 6, 12 and 18 months in high dose female rats and at 12 and 18 months in mid dose females.

Since the effect on ALP was consistently strong (>50%) throughout the study in high dose females and is in line with the other glyphosate the studies the effect is considered to be treatment related and adverse.

No other dose or treatment related significant changes were observed biochemical parameters. These changes observed were only minor and were not consistently seen at all sampling periods throughout the study.

Table B.6.5.5-4 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	**Males**				**Females**			
Group #	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
6th Month								
ALT [IU/L]	33 ± 4.87	37 ± 6.96	37 ± 4.21	33 ± 8.78	35 ± 5.12	32 ± 3.92	35 ± 7.32	38 ± 5.34
AST [IU/L]	70 ± 5.16	**78* ± 11.1**	**84* ± 14.9**	**89* ± 20.6**	85 ± 17.8	84 ± 14.6	80 ± 11.8	82 ± 8.58
Alp [IU/L]	213 ± 99.2	251 ± 61.8	227 ± 77.6	185 ± 30.6	133 ± 42.6	146 ± 39.2	152 ± 58.2	**235* ± 117.3 (+108%)**
GGT [IU/L]	5.8 ± 2.68	6.9 ± 3.42	7.2 ± 2.68	8.5 ± 3.35	7.1 ± 1.72	7.4 ± 3.13	6.0 ± 3.25	9.5 ± 3.20
Total Protein [g/dL]	8.1 ± 0.43	8.2 ± 0.30	8.1 ± 0.26	8.0 ± 0.60	8.2 ± 0.52	7.8 ± 0.65	**7.7* ± 0.22**	7.7 ± 0.63

Table B.6.5.5-4 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	**Males**				**Females**			
Group #	**G1**	**G2**	**G3**	**G4**	**G1**	**G2**	**G3**	**G4**
Dose (ppm)	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Alb [g/dL]	4.0 ± 0.17	3.9 ± 0.26	4.0 ± 0.33	3.9 ± 0.19	3.7 ± 0.18	3.7 ± 0.55	3.7 ± 0.16	**3.5* ± 0.19**
BUN [mg/dL]	17 ± 1.2	16 ± 2.0	**14* ± 1.2**	**15* ± 1.8**	19 ± 1.5	**16* ± 2.0**	18 ± 1.4	18 ± 2.0
Glu [mg/dL]	117 ± 8.78	114 ± 12.6	118 ± 15.9	124 ± 16.0	113 ± 6.75	108 ± 9.01	116 ± 8.55	117 ± 10.4
12th Month								
ALT [IU/L]	49 ± 15.3	47 ± 14.2	42 ± 11.5	50 ± 11.4	29 ± 9.68	31 ± 7.44	38 ± 15.7	34 ± 11.4
AST [IU/L]	77 ± 12.6	77 ± 12.0	72 ± 11.4	73 ± 10.4	75 ± 10.6	84 ± 7.55	79 ± 20.7	74 ± 10.4
Alp [IU/L]	244 ± 82.3	228 ± 101.5	298 ± 69.4	128 ± 76.9	141 ± 34.2	158 ± 34.1	**203* ± 70.4**	**231* ± 74.6** (+64%)
GGT [IU/L]	8.3 ± 2.06	8.3 ± 4.09	8.4 ± 3.50	**5.1* ± 2.46**	5.8 ± 2.20	7.7 ± 3.24	6.3 ± 2.68	5.3 ± 2.18
Total Protein [g/dL]	9.2 ± 0.46	9.1 ± 0.32	9.3 ± 0.58	**8.7* ± 0.34**	8.3 ± 0.98	8.0 ± 0.59	8.4 ± 0.78	8.5 ± 0.94
Alb [g/dL]	3.9 ± 0.23	3.9 ± 0.27	**4.3* ± 0.34**	4.2 ± 0.40	3.7 ± 0.21	**3.5* ± 0.09**	3.5 ± 0.07	3.5 ± 0.10
BUN [mg/dL]	18 ± 1.1	18 ± 1.8	19 ± 2.5	18 ± 1.5	14 ± 2.4	15 ± 1.9	14 ± 1.6	13 ± 2.1
Glu [mg/dL]	130 ± 14.8	**115* ± 12.3**	**111* ± 14.2**	**114* ± 8.72**	117 ± 11.3	113 ± 5.14	117 ± 6.80	120 ± 13.9
18th Month								
ALT [IU/L]	73 ± 15.0	85 ± 24.1	67 ± 22.2	75 ± 8.06	59 ± 13.5	68 ± 18.2	77 ± 24.2	**79* ± 14.7**
AST [IU/L]	117 ± 17.2	142 ± 42.8	118 ± 24.3	115 ± 27.0	105 ± 15.9	131 ± 43.7	122 ± 55.5	**125* ± 25.1**
Alp [IU/L]	211 ± 90.5	289 ± 159	188 ± 77.5	174 ± 49.5	101 ± 36.7	139 ± 60.6	**197* ± 105**	**194* ± 86.3 (+92%)**
GGT [IU/L]	8.4 ± 4.44	10.4 ± 2.78	8.98 ± 3.43	6.9 ± 3.15	5.3 ± 2.29	7.1 ± 1.40	8.4 ± 4.28	7.6 ± 3.59
Total Protein [g/dL]	7.6 ± 0.28	7.5 ± 0.63	7.6 ± 0.50	7.4 ± 0.50	8.0 ± 0.46	8.0 ± 0.48	8.0 ± 0.57	7.8 ± 0.49
Alb [g/dL]	3.1 ± 0.18	**2.7* ± 0.24**	3.2 ± 0.32	3.1 ± 0.21	3.8 ± 0.13	**3.5* ± 0.16**	**3.6* ± 0.27**	3.6 ± 0.29
BUN [mg/dL]	19 ± 1.67	20 ± 2.38	17 ± 1.40	19 ± 2.01	18 ± 2.02	18 ± 2.86	21 ± 3.38	20 ± 2.23
Glu [mg/dL]	114 ± 8.13	**104* ± 12.9**	103 ± 15.5	**105* ± 8.46**	124 ± 11.1	122 ± 20.4	**111* ± 13.4**	117 ± 5.62
24th Month								
ALT [IU/L]	97 ± 39.7	85 ± 20.3	270 ± 455	103 ± 29.6	65 ± 23.5	72 ± 22.2	69 ± 45.2	73 ± 20.5
AST [IU/L]	128 ± 57.7	130 ± 28.8	226 ± 269	111 ± 11.1	153 ± 126	138 ± 70.7	175 ± 220	155 ± 146
Alp [IU/L]	261 ± 68.4	**351* ± 80.7**	281 ± 177	292 ± 104	254 ± 117	274 ± 109	220 ± 70.1	249 ± 88.3
GGT [IU/L]	6.8 ± 2.59	5.3 ± 2.95	19.1 ± 44.2	6.5 ± 11.8	15.1 ± 33.9	5.1 ± 3.18	4.1 ± 6.77	2.5 ± 1.18
Total Protein [g/dL]	8.0 ± 0.53	8.2 ± 0.50	7.6 ± 0.34	8.0 ± 0.27	8.2 ± 0.52	8.6 ± 0.34	8.2 ± 0.86	**7.4* ± 0.36**

Table B.6.5.5-4 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Clinical chemistry data

Sex	Males				Females			
Group #	G1	G2	G3	G4	G1	G2	G3	G4
Dose (ppm)	0	100	1000	10000	0	100	1000	10000
Alb [g/dL]	2.8 ± 0.27	2.7 ± 0.13	2.7 ± 0.16	2.7 ± 0.28	3.3 ± 0.3	3.4 ± 0.35	3.1 ± 0.30	**3.1*** ± **0.16**
BUN [mg/dL]	18 ± 2.95	18 ± 1.65	17 ± 2.78	19 ± 2.27	17 ± 1.60	16 ± 1.91	18 ± 3.16	16 ± 1.96
Glu [mg/dL]	129 ± 13.0	122 ± 12.4	116 ± 30.0	**105*** ± **13.1**	130 ± 16.5	117 ± 16.7	**110*** ± **9.24**	129 ± 7.71

* Statistically significant ($p \leq 0.05$)

G. URINALYSIS

There were no treatment-related findings.

H. NECROPSY

Gross pathology

The incidence of liver lesions was increased in the high dose group males and females, consisting of small livers, focal haemorrhage, small cyst and a pale and mottled appearance. Three small mesenteric masses were seen in the males and females of the high dose group. In the lungs increased incidence of emphysema; collapse; petechiae, ecchymoses were observed in high dose animals with the highest incidences in males. Number of animals showing gross pathology changes was higher in high dose group compared to control group, this was especially due to findings in liver and lungs.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Pathology and Histopathology

None of the significant microscopic changes, both increased and decreased incidences (spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed have shown dose relationship, hence appeared to be incidental and not related to the treatment with the test compound.

Cataract frequency was also comparable between control and dosing groups. Single incidences in the high dose group were higher than in the control group. However, when taking into account the physical examination of the eye six control animals showed cataracts, meaning that 3 animals with cataract have not been evaluated at necropsy (possibly due to autolysis). Taking into account the physical examination no difference between control and dose group was observed. In addition, when taking into account damaged eye, cataract and corneal opacity no difference between control and dose groups was observed.

Table B.6.5..5-5 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of findings in the eyes – all animals

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Necropsy findings in all rats								
No. of rats examined	50	50	50	50	50	50	50	50
Damaged	0	0	2	0	5	0	0	1
Cataract	3	4	2	7	1	4	5	4
Corneal opacity	4	1	4	2	2	4	2	2
Sum of eye findings listed above	7	5	8	9	8	8	7	7

Table B.6.5..5-5 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of findings in the eyes – all animals

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Damaged [%]	0	0	4	0	10	0	0	2
Cataract [%]	6	8	4	14	2	8	10	8
Corneal opacity [%]	8	2	8	4	4	8	4	4
Sum of eye findings listed above [%]	14	10	16	18	16	16	14	14

Neoplastic changes

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 by individual sex or for combined sex.

Table B.6.5..5-6 Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats ([redacted] 1996): Summary of neoplastic histopathological findings (dead and moribund sacrificed animals)

	Dose group (ppm)							
	Males				Females			
	G1 0	G2 100	G3 1000	G4 10000	G1 0	G2 100	G3 1000	G4 10000
Dead and moribund sacrificed animals								
Liver: Cholangio carcinoma	0/30	2/30	2/32	2/21	1/26	0/23	0/17	0/29
Liver: Hepatocellular adenoma	9/30	9/30	6/32	6/21	2/26	8/23	3/17	5/29
Liver: Hepatocellular carcinoma	12/30	12/30	9/32	5/21	4/26	4/23	2/17	5/29
Liver: Intrahepatic bile duct adenoma	1/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Liver: Histiocytic sarcoma	2/30	0/30	2/32	1/21	1/26	0/23	0/17	0/29
Liver: Tumour emboli	0/30	0/30	1/32	1/21	0/26	0/23	0/17	0/29
Liver: Fibrosarcoma	0/30	1/30	0/32	0/21	0/26	0/23	0/17	0/29
Mandibular lymph node: Lymphoma	0/29	0/29	0/32	0/21	0/26	0/24	0/17	0/29
Terminally sacrificed animals								
Liver: Cholangiocarcinoma	1/20	1/20	0/16	1/29	0/24	0/25	0/32	0/21
Liver: Hepatocellular adenoma	15/20	13/20	4/16	15/29	16/24	10/25	16/32	8/21
Liver: Hepatocellular carcinoma	9/20	16/20	9/16	19/29	6/24	11/25	12/32	4/21
Liver: Intrahepatic bile duct adenoma	1/20	0/20	0/16	0/29	0/24	1/25	0/32	0/21
Liver: Histocytic sarcoma	0/20	1/20	1/16	0/29	0/24	0/25	3/32	0/21
Liver: Tumour emboli	1/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21
Liver: Lymphosarcoma	1/20	0/20	0/16	0/29	0/24	0/25	0/32	0/21
Liver: Benign mixed intrahepatic bile duct adenoma	0/20	0/20	1/16	0/29	0/24	0/25	0/32	0/21
Mandibular lymph node: Lymphoma	0/19	0/6	0/5	2/29	0/24	0/9	0/6	0/21

Incidentally, the number of benign tumours in the low and mid dose group males and combined sex was lower and higher in the mid dose group females. There was no dose-response relationship and therefore the occurrence of tumours was considered incidental.

The different liver tumours observed in the dead and moribund sacrificed and terminally sacrificed rats included hepatocellular adenoma, intrahepatic bile duct adenomas, cholangiocarcinoma, hepatocellular carcinoma, histiocytic sarcoma, fibrosarcoma and lymphosarcoma. Of these, hepatocellular adenomas and carcinomas occurred more frequently, as often observed in ageing rats. The occurrence of these tumours appeared to be incidental and not compound-related as their frequency of occurrence was not dose dependent. No reasons could be ascribed for the decrease in the number of benign tumours in the low and mid dose group males and for combined sex and for an increase seen in the mid group dose females (see table above).

In addition, the AGG noted an apparent increase in the incidence of mandibular lymph node lymphoma in the high dose males at terminal sacrifice. The relevance of this finding in the context of the classification is discussed in Volume 1. ***The applicant is asked to provide historical control data for the effect on mandibular lymph node lymphoma, if available.***

Assessment and conclusion by applicant:
Based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 595 mg/kg bw/day for males, and 886 mg/kg bw/day for females (741 mg/kg bw/day for combined) for systemic toxicity and carcinogenicity. It is concluded that glyphosate technical is not carcinogenic in rats.

Single and inconsistent changes of clinical chemistry parameters were considered not treatment-related. (Isolated) Elevation of alkaline phosphatase activities is considered to be related to an adaptation of the metabolism rather than to damage of (liver) cells. Histopathology did not reveal any treatment-related changes in any organ further supporting the conclusion that clinical chemistry parameters changes were not related to treatment-related adverse effects.

Assessment and conclusion by RMS:
Based on the increase in cataract in high dose males and increase in ALP in high dose females the NOAEL is set at 1000 ppm (equal to 59.4 mg/kg bw/day in males and 88.5 mg/kg bw/day in females).

This NOAEL is in line with the evaluation made in the previous RAR (2015).

B.6.5.6. Long-term toxicity – rat, study 6

Data point:	CA 5.5/006
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: One Year Dietary Toxicity Study in Rats
Report No	[redacted]/P/5143
Document No	Not reported
Guidelines followed in study	OECD 452, US EPA 83-1
Deviations from current test guideline (OECD 452, 2018)	The following deviations from the current OECD guideline were noted : - Mortality was observed only once per day (instead of twice per day); - Blood samples were not taken at the beginning of the study (but in week 14); - Organ weights of heart, ovaries, spleen, thyroid/parathyroid and uterus were not determined. - Histopathological examination of the coagulating glands, lacrimal gland, mammary glands of the males and vagina was not performed.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid, Category 2a **Conclusion AGG:** Some deviations were noted compared to the current OECD test guideline. This mainly consisted of missing parameters in the organ weight measurements and histopathology which were added in the most recent version of the test Guideline. These deviations are not considered to be critical and therefore the study is concluded to be acceptable.

Full summary

The chronic toxicity potential of glyphosate acid was assessed in a 12-month feeding study in 24 male and female Wistar rats per group with 0, 2000, 8000 and 20000 ppm (equal to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).

Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as selected organ weights, necropsy and histopathological examination.

A reduction in bodyweight was evident in animals receiving 20000 ppm glyphosate acid. There were no toxicologically significant or treatment-related effects on haematology, urine clinical chemistry or organ weights. An increase in ALP was observed at all dose levels. The effect at 2000 ppm was only marginal and without accompanying pathological changes not considered to be adverse. Prostatitis was observed in high dose males and proliferative cholangitis of the liver in high dose females.

In addition, an increased incidence of mild focal basophilia of the acinar cells of the parotid salivary gland was observed in animals which had received 8000 (females only) and 20000 ppm (both sexes) glyphosate acid.

The NOAEL was concluded to be 2000 ppm (equal to 141 mg/kg bw/day in males and 167 mg/kg bw/day in females).

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Stability of test compound:	At least 1 year when stored at RT

2. Vehicle and/or positive control:

Diet

3. Test animals:

Species:	Rat
Strain:	Wistar (Alpk:APfSD)
Source:	[redacted]
Age:	22 - 24 days (on delivery)
Sex:	Males and females
Weight at dosing:	Males: 150.5 – 151.5 g (mean values); females: 126.7 – 133.3 g (mean values)

Acclimation period:	Approximately 2 weeks
Diet/Food:	CT1 diet (Special Diet services Ltd., Essex, UK), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	Initially in litters, sexes separately, after assignment to experimental groups in group of four rats per sex per cage.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15 % Air changes: at least 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-04-03 to 1996-06-03

Animal assignment and treatment:
In a chronic toxicity study groups of 24 Wistar-derived rats per sex received daily dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate acid (equivalent to mean achieved dose levels of 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females).

Test diets were prepared in either 30 or 60 kg batches by mixing the appropriate amount of the test substance with the basal diet. The stability and homogeneity of the test substance in the diet was determined in an in-house stability study at 2000 and 20000 ppm.

Observations
Rats were examined for toxic signs, ill-health or behavioural changes and pre-terminal deaths prior to the start of the study and once a day afterwards. Detailed clinical observations were conducted weekly. Ophthalmic examination was done in all animals at the start of the study. The eyes of the control and high dose group were additionally examined one week to termination.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 14 and every two weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from Week 1 to Week 13, once in Week 16 and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 12 animals per sex and group at Week 14, 27 and at termination (Week 53). The following parameters were measured:
Haematology : Haematocrit, haemoglobin, erythrocyte count, MCV, MCH, MCHC, blood cell morphology, platelet count, total leukocyte count, differential leukocyte count, red blood cell distribution width, prothrombin time, activated partial thromboplastin time.
Clinical chemistry : alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, urea, total protein, glucose, albumin, total bilirubin, triglycerides, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses (except for Week 52) at Week 13, 26 and 52. The following parameters were determined: Volume, colour, appearance, specific gravity, pH, glucose, ketones, protein, urobilinogen and blood.

Sacrifice and pathology

Necropsy was conducted on all animals except for Rats 38 and 149-152, which were killed during Week 6/7 due to a sexing error. The following organ weights were determined from all animals surviving to scheduled termination: Adrenals, brain, epididymis, kidneys, liver and testes.

Tissue samples were taken from the following organs: Adrenals, aorta, bone & bone marrow (femur incl. joint), brain (cerebrum, cerebellum, brainstem), caecum, cervix, colon, duodenum, epididymis, eye, gross lesions, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes (cervical and mesenteric), mammary gland, nasopharyngeal cavity, sciatic nerve, oesophagus, oral cavity, ovary, pancreas, pituitary, prostrate, rectum, salivary glands, seminal vesicles, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and voluntary muscle.

Statistics
All data were evaluated using analysis of variance and covariance for each specified parameter using the GLM procedure in SAS (1989). Differences from control were tested statistically by comparing each treatment group least- squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis. All statistical tests were two sided.

II. RESULTS

The mean achieved concentrations of glyphosate acid in each dietary preparation were within 8 % of the nominal concentration and the overall mean concentrations were within 4 % of nominal.

The homogeneity of glyphosate acid in diet at concentrations of 2000 and 20000 ppm was satisfactory; percentage deviations were within 7 % of the overall mean.

The stability tests determined at 2000 and 20000 ppm showed that the test substance is stable for at least 61 days when stored at room temperature.

A. MORTALITY AND CLINICAL SIGNS
There were no treatment-related deaths.

Table B.6.5.6-1 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Unscheduled deaths

Dose group (ppm)							
0		**2000**		**8000**		**20000**	
♂	♀	♂	♀	♂	♀	♂	♀
1/24	0/24	1/24	1/24	0/24	0/24	1/24	4/24

B. CLINICAL OBSERVATIONS
There was a small increase in the number of animals in the 20000 ppm group which had urinary staining (wet or dry). No other treatment related effects were observed.

Table B.6.5.6-2 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Selected clinical observations

	Dose group (ppm)							
	0		**2000**		**8000**		**20000**	
	♂	♀	♂	♀	♂	♀	♂	♀
	Number of observations/number of dead animals							
Stained with urine – dry	0/0	0/0	11/1	0/0	0/0	1/1	15/3	35/3
Stained with urine – wet	0/0	0/0	9/2	1/1	0/0	1/1	19/2	32/5

C. BODY WEIGHT

Bodyweights of rats receiving 20000 ppm glyphosate acid were lower than those of controls throughout the study. Bodyweights in the intermediate group were slightly reduced throughout the study. The difference from control was not statistically significant in males and was statistically significant in females only from Week 46. As the pattern of the effect was similar to that of the high dose rats for both sexes this minor difference in bodyweight is considered to be related to administration of glyphosate acid. However, since the effect is only slight (<10%) it is not considered to be adverse.

There was no effect on bodyweight in rats receiving 2000 ppm glyphosate acid.

The intergroup comparison of body weights are shown below.

Table B.6.5.6-3 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of body weights

	Dose group (ppm)							
	0		**2000**		**8000**		**20000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	151.5 ± 22.5 (N = 24)	128.5 ± 15.3 (N = 24)	↓150.5 ± 22.6 (N = 23)	↑130.3 ± 16.7 (N = 24)	↓150.6 ± 23.7 (N = 24)	↓126.7 ± 15.1 (N = 24)	↓151.0 ± 21.1 (N = 24)	↓128.1 ± 17.0 (N = 20)
Week 2 adjusted mean	204.1 ± 24.1 (N = 24) 203.3	158.0 ± 12.0 (N = 24) 158.0	↓202.2 ± 23.4 (N = 23) 202.6	↑158.7 ± 12.4 (N = 24) 156.5	↓202.7 ± 23.7 (N = 24) 203.1	↓153.6 ± 12.7 (N = 24) 155.6	**↓198.2 ± 22.0 (N = 24) 198.1** (-3%)**	**↓151.9 ± 16.7 (N = 20) 151.7** (-4%)**
Week 3 adjusted mean	252.7 ± 25.3 (N = 23) 253.5	179.5 ± 11.2 (N = 24) 179.4	↑254.4 ± 27.3 (N = 23) 254.5	↑180.8 ± 15.0 (N = 24) 178.5	↑252.9 ± 30.8 (N = 24) 252.7	↓175.6 ± 14.1 (N = 24) 177.6	**↓245.0 ± 22.8 (N = 24) 244.3** (-4%)**	↓176.8 ± 14.8 (N = 20) 176.7 (-2%)
Week 4 adjusted mean	301.3 ± 36.8 (N = 24) 300.3	197.9 ± 10.8 (N = 24) 197.8	↑297.7 ± 26.9 (N = 23) 298.4	↑202.2 ± 12.5 (N = 24) 200.1	↓295.7 ± 30.1 (N = 24) 296.1	↓192.1 ± 14.8 (N = 24) 194.0	**↓284.5 ± 23.1 (N = 24) 284.4** (-5%)**	**↓189.3 ± 15.8 (N = 20) 188.9** (-5%)**
Week 5 adjusted mean	322.0 ± 27.5 (N = 24) 331.1	213.8 ± 11.0 (N = 24) 213.7	↑330.9 ± 28.1 (N = 23) 331.6	↑216.1 ± 13.2 (N = 24) 214.1	↑326.3 ± 30.4 (N = 24) 326.8	↓208.2 ± 13.8 (N = 24) 210.0	**↓312.2 ± 23.9 (N = 24) 312.1** (-6%)**	**↓203.5 ± 13.4 (N = 20) 203.2** (-5%)**
Week 6 adjusted mean	357.4 ± 30.7 (N = 24) 356.3	224.0 ± 12.6 (N = 24) 223.9	↑357.7 ± 29.8 (N = 23) 358.7	↑224.4 ± 14.9 (N = 24) 222.3	↓350.2 ± 33.1 (N = 24) 350.7	↓217.5 ± 15.8 (N = 24) 219.4	**↓333.8 ± 28.3 (N = 23) 333.7** (-6%)**	**↓215.6 ± 13.8 (N = 20) 215.4* (-4%)**
Week 12 adjusted mean	473.0 ± 39.9 (N = 24) 471.5	266.3 ± 13.8 (N = 24) 266.2	↑474.0 ± 38.7 (N = 23) 475.4	↑269.3 ± 13.3 (N = 24) 267.1	↓456.5 ± 45.0 (N = 24) 457.2	↓258.8 ± 17.8 (N = 24) 260.8	**↓441.0 ± 38.8 (N = 24) 440.8** (-7%)**	**↓256.3 ± 17.0 (N = 20) 261.2** (-2%)**
Week 24 adjusted mean	566.2 ± 49.3 (N = 24) 564.3	296.2 ± 12.7 (N = 24) 296.1	↓560.3 ± 48.3 (N = 23) 561.4	↑301.5 ± 14.8 (N = 24) 299.6	↓540.7 ± 55.1 (N = 24) 541.5*	↓287.8 ± 19.9 (N = 24) 289.4	**↓531.9 ± 46.3 (N = 24) 531.7** (-6%)**	**↓287.1 ± 18.7 (N = 20) 286.9* (-3%)**

Table B.6.5.6-3 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of body weights

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 36 adjusted mean	624.5 ± 50.5 (N = 24) 622.5	316.2 ± 19.7 (N = 24) 316.1	↓613.9 ± 52.8 (N = 23) 614.7	↑319.0 ± 17.3 (N = 24) 316.8	↓599.6 ± 61.9 (N = 24) 613.0	↓307.8 ± 25.2 (N = 24) 309.7	**↓593.9 ± 57.6** **(N = 24)** **593.7*** **(-5%)**	↓305.4 ± 36.2 (N = 20) 304.4
Week 53 adjusted mean	652.1 ± 53.0 (N = 23) 548.5	↓346.8 ± 26.9 (N = 24) 346.8	↓644.0 ± 56.8 (N = 23) 643.2	↓344.1 ± 26.5 (N = 23) 339.3	↓640.5 ± 62.7 (N = 24) 640.1	**↓327.7 ± 30.2** **(N = 24)** **330.8***	↓634.1 ± 60.1 (N = 23) 634.3	↓332.5 ± 40.5 (N = 20) 330.6

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

D. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was generally lower in rats receiving 20000 ppm than in controls. The difference was most marked at the start of the study. Food consumption was generally slightly lower than controls in rats receiving 8000 ppm glyphosate acid. There was no effect on food consumption in rats receiving 2000 ppm.

Table B.6.5.6-4 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of food consumption

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]								
Week 1	26.3 ± 2.0	21.0 ± 1.3	↓25.9 ± 1.7	↓20.9 ± 1.3	↓25.8 ± 2.2	↓20.3 ± 0.9	↓25.1 ± 1.8	**↓19.5* ± 1.3 (-7%)**
Week 2	28.7 ± 2.1	21.0 ± 0.7	↓28.5 ± 1.7	↓20.9 ± 0.9	↓28.2 ± 2.0	↑21.6 ± 2.5	**↓27.2* ± 1.2 (-5%)**	↓20.7 ± 0.8
Week 3	30.6 ± 1.7	21.4 ± 0.9	↓30.1 ± 0.8	↑21.9 ± 0.9	↓29.8 ± 1.1	↓20.6 ± 1.2	**↓28.5** ± 1.1 (-7%)**	**↓20.1* ± 0.8 (-6%)**
Week 4	30.6 ± 1.7	21.7 ± 0.2	↓30.3 ± 0.9	21.7 ± 0.7	↓29.4 ± 1.4	↓20.9 ± 1.3	**↓28.0** ± 2.0 (-8%)**	**↓19.8** ± 0.5 (-9%)**
Week 5	30.7 ± 1.7	22.3 ± 0.7	↓30.5 ± 1.6	↓21.7 ± 0.9	↓29.8 ± 1.7	**↓21.0* ± 1.2**	**↓27.8** ± 2.0 (-9%)**	**↓20.5** ± 0.4 (-8%)**
Week 6	30.8 ± 1.7	22.0 ± 0.6	↓30.4 ± 1.6	↓21.7 ± 1.1	↓29.9 ± 1.1	↓21.0 ± 1.2	**↓28.1** ± 1.5 (-9%)**	**↓20.7* ± 1.7 (-6%)**
Week 12	31.2 ± 1.9	22.0 ± 0.9	↑31.3 ± 2.0	↓21.4 ± 0.7	**↓29.8* ± 2.0**	**↓20.8* ± 0.9**	**↓29.9* ± 1.1 (-4%)**	**↓20.5* ± 0.8 (-7%)**
Week 24	30.4 ± 1.4	20.6 ± 0.9	↓29.8 ± 1.5	↓20.2 ± 0.9	↓29.3 ± 1.4	↓20.2 ± 1.4	↓29.6 ± 0.7	↓20.1 ± 1.1
Week 36	28.8 ± 1.2	20.8 ± 1.7	↑29.0 ± 1.5	↓20.2 ± 2.1	↓28.3 ± 1.4	↓19.8 ± 1.5	↓28.4 ± 0.6	**↓19.6* ± 1.3**

Table B.6.5.6-4 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of food consumption

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
								(-6%)
Week 52	26.4 ± 1.1	19.9 ± 1.2	↑26.7 ± 2.0	↓19.6 ± 2.0	↑26.8 ± 0.9	↓19.3 ± 1.7	↓25.9 ± 0.2	↓19.4 ± 0.6

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

The group mean achieved doses are summarised below.

Table B.6.5.6-5 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
1 (control)	0		
2 (low)	2000	141	167
3 (mid)	8000	560	671
4 (high)	20000	1409	1664

The results show a higher test material intake for females when compared to males for each dose level. The mean intake for each dose group is 0, 141, 560 and 1409 mg/kg bw/day for males and 0, 167, 671 and 1664 mg/kg bw/day for females for 0, 2000, 6000 and 20000 ppm, respectively.

E. OPHTHALMOSCOPY

There were no treatment-related effects observed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

A number of statistically significant differences from control were identified but there was no evidence of a relationship to dose and the differences were small and not seen consistently at all the time points and therefore were considered to be unrelated to glyphosate acid administration. All statistical findings are included in the table below.

Table B.6.5.6-6 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Haematocrit [g/dL]								
Week 14	0.460 ± 0.014 (N = 12)	0.451 ± 0.009 (N = 12)	↓0.455 ± 0.020 (N = 12)	↓0.444 ± 0.013 (N = 12)	0.460 ± 0.016 (N = 12)	↓0.446 ± 0.020 (N = 11)	↓0.459 ± 0.012 (N = 12)	0.451 ± 0.014, (N = 10)
Week 27	0.450 ± 0.018 (N = 11)	0.436 ± 0.022 (N = 10)	↓0.447 ± 0.017 (N = 12)	↑0.442 ± 0.019 (N = 12)	↑0.451 ± 0.021 (N = 12)	↑0.449 ± 0.016 (N = 12)	↓0.444 ± 0.013 (N = 12)	↑0.445 ± 0.014 (N = 10)
Week 53	0.436 ± 0.022	0.443 ± 0.019	↑0.438 ± 0.020	↑0.448 ± 0.023	**↑0.450* ± 0.021**	↑0.445 ± 0.020	↑0.440 ± 0.018	↑0.444 ± 0.019

Table B.6.5.6-6 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	(N = 23)	(N = 24)	(N = 23)	(N = 23)	**(N = 24)**	(N = 24)	(N = 23)	(N = 20)
Mean cell volume [fL]								
Week 14	49.8 ± 1.7 (N = 12)	54.9 ± 1.4 (N = 12)	↑50.2 ± 0.8 (N = 12)	↓**53.7* ± 1.3 (N = 12)**	↑50.7 ± 1.1 (N = 12)	↓**53.5** ± 1.5 (N = 12)**	↑50.3 ± 1.3 (N = 12)	↓**53.6* ± 1.3 (N = 10)**
Week 27	48.3 ± 2.2 (N = 11)	53.9 ± 2.0 (N = 10)	↑48.9 ± 1.2 (N = 12)	↓53.4 ± 1.9 (N = 12)	↑**49.5** ± 1.7 (N = 12)**	↓53.7 ± 1.7 (N = 12)	↑48.6 ± 1.0 (N = 12)	↓53.1 ± 1.5 (N = 10)
Week 53	51.0 ± 2.0 (N = 23)	55.4 ± 2.3 (N = 24)	↑51.2 ± 1.7 (N = 23)	↑55.5 ± 2.0 (N = 23)	↑51.8 ± 2.0 (N = 24)	↓54.9 ± 2.0 (N = 24)	↓50.8 ± 1.5 (N = 23)	↓54.8 ± 1.8 (N = 20)
Mean cell haemoglobin [pg]								
Week 14	17.3 ± 0.6 (N = 12)	19.2 ± 0.4 (N = 12)	↑17.6 ± 0.3 (N = 12)	↓18.9 ± 0.4 (N = 12)	↑**17.7* ± 0.4 (N = 12)**	↓18.9 ± 0.5 (N = 11)	↑**17.7* ± 0.5 (N = 12)**	↓**18.7* ± 0.4 (N = 10)**
Week 27	16.7 ± 0.6 (N = 11)	18.9 ± 0.5 (N = 10)	↑17.0 ± 0.4 (N = 12)	↓18.8 ± 0.5 (N = 12)	↑**17.2** ± 0.4 (N = 12)**	↓18.8 ± 0.5 (N = 12)	↑17.0 ± 0.5 (N = 12)	↓18.6 ± 0.4 (N = 10)
Week 53	16.9 ± 0.5 (N = 23)	18.4 ± 0.5 (N = 24)	16.9 ± 0.4 (N = 23)	↓18.3 ± 0.5 (N = 23)	↑17.0 ± 0.7 (N = 24)	18.4 ± 0.5 (N = 24)	↓16.8 ± 0.5 (N = 23)	18.4 ± 0.4 (N = 20)
Mean cell haemoglobin concentration [g/dL]								
Week 14	34.8 ± 0.6 (N = 12)	35.0 ± 0.2 (N = 12)	↑35.0 ± 0.4 (N = 12)	↑35.2 ± 0.5 (N = 12)	↑35.0 ± 0.5 (N = 12)	↑**35.3* ± 0.5 (N = 11)**	↑35.1 ± 0.3 (N = 12)	35.0 ± 0.5 (N = 10)
Week 27	34.6 ± 0.7 (N = 11)	35.1 ± 0.8 (N = 10)	↑34.8 ± 0.5 (N = 12)	↑35.2 ± 0.7 (N = 12)	↑34.7 ± 0.9 (N = 12)	↓34.9 ± 0.6 (N = 12)	↑34.9 ± 0.4 (N = 12)	↓35.0 ± 0.7 (N = 10)
Week 53	33.1 ± 0.6 (N = 23)	33.2 ± 1.0 (N = 24)	↓33.0 ± 0.8 (N = 23)	↓33.1 ± 0.9 (N = 23)	↓32.8 ± 1.0 (N = 24)	↑33.5 ± 0.9 (N = 24)	↑33.1 ± 0.5 (N = 23)	↑33.6 ± 0.9 (N = 20)
Red cell distribution width [%]								
Week 14	13.4 ± 0.6 (N = 12)	11.9 ± 1.2 (N = 12)	↓13.1 ± 0.6 (N = 12)	↑12.0 ± 0.7 (N = 12)	↑13.6 ± 1.3 (N = 12)	↓11.7 ± 0.9 (N = 11)	13.4 ± 0.8 (N = 12)	↓11.5 ± 0.7 (N = 10)
Week 27	15.1 ± 0.6 (N = 11)	12.6 ± 0.4 (N = 10)	↓**14.6** ± 0.6 (N = 12)**	↑**13.0* ± 0.5 (N = 12)**	↓14.8 ± 0.7 (N = 12)	↑**13.0* ± 0.6 (N = 12)**	↓**14.6** ± 0.6 (N = 12)**	↑12.7 ± 0.4 (N = 10)
Week 53	14.2 ± 0.5 (N = 23)	12.4 ± 0.6 (N = 24)	↓13.9 ± 0.7 (N = 23)	↓12.3 ± 0.4 (N = 23)	↓14.0 ± 0.4 (N = 24)	12.4 ± 0.8 (N = 24)	↓14.0 ± 0.6 (N = 23)	↓12.2 ± 0.6 (N = 20)
Platelet count [x109/1]**								
Week 14	857 ± 84 (N = 12)	768 ± 98 (N = 12)	↓849 ± 78 (N = 12)	↑804 ± 80 (N = 12)	↓850 ± 61 (N = 12)	↓757 ± 82 (N = 12)	↓825 ± 66 (N = 12)	↑818 ± 97 (N = 10)
Week 27	893 ± 56 (N = 11)	785 ± 158 (N = 10)	↓866 ± 110 (N = 12)	↓782 ± 110 (N = 12)	↑915 ± 82 (N = 12)	↓780 ± 94 (N = 12)	↓867 ± 150 (N = 12)	↑846 ± 139 (N = 10)
Week 53	917 ± 87 (N = 23)	822 ± 104 (N = 24)	↑939 ± 121 (N = 23)	↓782 ± 102 (N = 23)	↑**962* ± 109 (N = 24)**	↓776 ± 98 (N = 24)	917 ± 107 (N = 23)	↑868 ± 83 (N = 20)
Neutrophil count [x109/1]**								

Table B.6.5.6-6 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Intergroup comparison of haematology

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 14	1.49 ± 0.51 (N = 12)	1.20 ± 0.45 (N = 12)	↑1.58 ± 0.35 (N = 12)	↑1.61 ± 1.38 (N = 12)	↑1.35 ± 0.38 (N = 12)	↓1.06 ± 0.35 (N = 12)	↑1.32 ± 0.32 (N = 12)	↓0.99 ± 0.32 (N = 10)
Week 27	2.08 ± 0.72 (N = 11)	1.23 ± 0.51 (N = 10)	↑2.13 ± 0.74 (N = 12)	↑1.44 ± 0.34 (N = 12)	↑1.85 ± 0.56 (N = 12)	↓1.20 ± 0.60 (N = 12)	↑1.94 ± 0.51 (N = 12)	↓1.11 ± 0.30 (N = 10)
Week 53	2.11 ± 0.80 (N = 23)	1.29 ± 0.43 (N = 24)	↓1.99 ± 0.67 (N = 23)	↑1.37 ± 0.47 (N = 23)	**↑1.39** ± 0.36 (N = 24)**	↑1.36 ± 0.49 (N = 24)	**↑1.80* ± 0.75 (N = 23)**	↓1.14 ± 0.43 (N = 20)
Monocyte count [x109/l]**								
Week 14	0.130 ± 0.051 (N = 12)	0.101 ± 0.027 (N = 12)	↓0.128 ± 0.033 (N = 12)	↑0.112 ± 0.066 (N = 12)	↓0.119 ± 0.038 (N = 12)	↓0.090 ± 0.035 (N = 12)	↑0.142 ± 0.035 (N = 12)	↓0.091 ± 0.026 (N = 10)
Week 27	0.181 ± 0.087 (N = 11)	0.118 ± 0.047 (N = 10)	**↑0.240* ± 0.106 (N = 12)**	↑0.138 ± 0.083 (N = 12)	↑0.207 ± 0.094 (N = 12)	↑0.121 ± 0.085 (N = 12)	↑0.221 ± 0.097 (N = 12)	↓0.113 ± 0.041 (N = 10)
Week 53	0.209 ± 0.100 (N = 23)	0.194 ± 0.102 (N = 24)	↑0.232 ± 0.135 (N = 23)	↑0.234 ± 0.156 (N = 23)	↑0.227 ± 0.154 (N = 24)	↓0.180 ± 0.109 (N = 24)	↑0.226 ± 0.138 (N = 23)	↓0.172 ± 0.109 (N = 20)
Eosinophil count [x109/l]**								
Week 14	0.222 ± 0.064 (N = 12)	0.164 ± 0.083 (N = 12)	↓0.200 ± 0.030 (N = 12)	↓0.155 ± 0.052 (N = 12)	↓0.207 ± 0.071 (N = 12)	**↓0.114* ± 0.028 (N = 12)**	↑0.245 ± 0.095 (N = 12)	↓0.124 ± 0.052 (N = 10)
Week 27	0.268 ± 0.123 (N = 11)	0.145 ± 0.041 (N = 10)	↓0.248 ± 0.077 (N = 12)	↑0.158 ± 0.065 (N = 12)	↓0.238 ± 0.076 (N = 12)	↓0.126 ± 0.049 (N = 12)	↓0.245 ± 0.095 (N = 12)	↓0.115 ± 0.027 (N = 10)
Week 53	0.187 ± 0.145 (N = 23)	0.092 ± 0.031 (N = 24)	↓0.156 ± 0.064 (N = 23)	↑0.113 ± 0.102 (N = 23)	**↓0.141* ± 0.028 (N = 24)**	↑0.108 ± 0.056 (N = 24)	↓0.161 ± 0.052 (N = 23)	↓0.072 ± 0.029 (N = 20)

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

Clinical chemistry
Plasma cholesterol and plasma triglycerides were marginally reduced in males receiving 20000 or 8000 ppm at Weeks 14 and 27. Bilirubin was increased in males receiving 20000 or 8000 ppm at week 14 and 53.

Moreover, there was a treatment- and dose-related increase in plasma ALP activity throughout the study. For rats receiving 2000 ppm glyphosate acid the increase was marginal (<50%) and was statistically significant only for females at Week 14 and therefore not considered to be adverse.

All other differences from control were small and/or were not dose-related and are considered to be incidental to administration of glyphosate acid. For example, plasma creatinine was reduced at all dose levels in males at 14 and 27 weeks but no dose-response was observed.

Table B.6.5.6-7 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma creatinine [µmol/L]								

Table B.6.5.6-7 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 14	102.1 ± 90.0 (N = 12)	61.8 ± 3.0 (N = 12)	↓**61.8** ± 2.3 (N = 11)**	↓60.7 ± 3.9 (N = 12)	↓**60.6** ± 3.6 (N = 11)**	↑62.0 ± 3.2 (N = 12)	↓**60.7** ± 2.5 (N = 12)**	↓60.8 ± 2.8 (N = 10)
Week 27	66.8 ± 6.0 (N = 12)	65.3 ± 4.1 (N = 12)	↓**64.4* ± 5.0 (N = 11)**	↓64.4 ± 3.1 (N = 12)	↓**61.1** ± 4.7 (N = 12)**	65.3 ± 2.6 (N = 12)	↓**63.4** ± 3.6 (N = 12)**	↓63.6 ± 4.0 (N = 10)
Week 53	62.3 ± 5.2 (N = 23)	57.9 ± 5.6 (N = 24)	↑62.9 ± 6.7 (N = 23)	↑58.8 ± 4.8 (N = 23)	↑63.2 ± 8.9 (N = 24)	↓55.2 ± 5.0 (N = 24)	↓59.6 ± 3.9 (N = 23)	↓55.8 ± 5.2 (N = 20)
Plasma cholesterol [mmol/L]								
Week 14	2.46 ± 0.21 (N = 12)	2.13 ± 0.30 (N = 12)	↑2.53 ± 0.30 (N = 11)	↑2.28 ± 0.29 (N = 12)	↓2.31 ± 0.31 (N = 11)	↑2.26 ± 0.28 (N = 12)	↓**2.28* ± 0.27 (N = 12) (-7%)**	↑2.21 ± 0.17 (N = 10)
Week 27	3.09 ± 0.40 (N = 12)	2.62 ± 0.38 (N = 12)	↓3.05 ± 0.47 (N = 11)	↑2.67 ± 0.39 (N = 12)	↓**2.75* ± 0.32 (N = 12)**	↑2.76 ± 0.34 (N = 12)	↓**2.70** ± 0.30 (N = 12) (-13%)**	↑2.78 ± 0.44 (N = 10)
Week 53	4.53 ± 1.15 (N = 23)	3.15 ± 0.97 (N = 24)	↑4.64 ± 1.35 (N = 23)	↓2.84 ± 0.63 (N = 23)	↑4.57 ± 2.08 (N = 24)	3.15 ± 0.59 (N = 24)	↓4.07 ± 1.36 (N = 23)	↑3.22 ± 0.41 (N = 20)
Plasma triglycerides [mmol/L]								
Week 14	1.56 ± 0.34 (N = 12)	0.94 ± 0.19 (N = 12)	↑1.63 ± 0.26 (N = 11)	↓0.92 ± 0.34 (N = 12)	↓**1.28** ± 0.21 (N = 11)**	↓0.89 ± 0.24 (N = 12)	↓**1.28** ± 0.37 (N = 12) (-18%)**	↑0.95 ± 0.21 (N = 10)
Week 27	1.51 ± 0.42 (N = 12)	1.07 ± 0.29 (N = 12)	↓1.43 ± 0.31 (N = 11)	↑1.10 ± 0.30 (N = 12)	↓**1.15** ± 0.22 (N = 12)**	↑1.13 ± 0.29 (N = 12)	↓**0.97** ± 0.24 (N = 12) (-36%)**	↑1.10 ± 0.41 (N = 10)
Week 53	1.70 ± 0.54 (N = 23)	1.11 ± 0.54 (N = 24)	↑1.74 ± 0.60 (N = 23)	↓0.92 ± 0.20 (N = 23)	↑1.74 ± 0.92 (N = 24)	↓0.99 ± 0.28 (N = 24)	↑1.78 ± 0.83 (N = 23)	↑1.21 ± 0.54 (N = 20)
Plasma total bilirubin [mmol/L]								
Week 14	1.92 ± 0.51 (N = 12)	1.83 ± 0.58 (N = 12)	↑2.18 ± 0.60 (N = 11)	↑1.92 ± 0.29 (N = 12)	↑2.18 ± 0.87 (N = 11)	↑2.08 ± 0.51 (N = 12)	↑**2.33* ± 0.65 (N = 12) (+21%)**	↑2.10 ± 0.57 (N = 10)
Week 27	2.17 ± 0.83 (N = 12)	2.17 ± 0.72 (N = 12)	↑2.18 ± 0.40 (N = 11)	↓**1.75* ± 0.45 (N = 12)**	↑2.33 ± 0.65 (N = 12)	↑2.25 ± 0.45 (N = 12)	↑2.50 ± 0.80 (N = 12)	↓2.10 ± 0.57 (N = 10)
Week 53	2.04 ± 0.56 (N = 23)	2.46 ± 0.59 (N = 24)	↓2.00 ± 0.30 (N = 23)	↑2.48 ± 0.79 (N = 23)	↓1.83 ± 0.48 (N = 24)	↑2.58 ± 0.65 (N = 24)	↑**2.43* ± 0.84 (N = 23) (+19%)**	↑2.65 ± 0.67 (N = 20)
Plasma alkaline phosphatase (IU/L)								
Week 14	248 ± 35 (N = 12)	161 ± 47 (N = 12)	↑281 ± 47 (N = 11)	↑**201 ± 71* (N = 12)**	↑**342 ± 67** (N = 11) (+38%)**	↑**227 ± 31** (N = 12) (+41%)**	↑**429 ± 85** (N = 12) (+73%)**	↑**292 ± 69** (N = 10) (+81%)**
Week 27	221 ± 38 (N = 12)	135 ± 45 (N = 12)	↑250 ± 37	↑171 ± 81	↑**306 ± 55****	↑**200 ± 39****	↑**412 ± 108****	↑**254 ± 66****

Table B.6.5.6-7 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
			(N = 11)	(N = 12)	**(N = 12) (+38%)**	**(N = 12) (+48%)**	**(N = 12) (+86%)**	**(N = 10) (+88%)**
Week 53	232 ± 89 (N = 23)	87 ± 42 (N = 12)	↑258 ± 61 (N = 23)	↑100 ± 34 (N = 12)	**↑291 ± 54** (N =24) (+25%)**	↑114 ± 38 (N = 24) (+31%)	**↑379 ± 101** (N = 23) (+63%)**	**↑160 ± 77** (N = 19) (+83%)**
Plasma gamma-glutamyl transferase (IU/L)								
Week 14	1.5 ± 1.2 (N = 12)	0.8 ± 0.9 (N = 12)	↓1.1 ± 0.8 (N = 11)	↑1.1 ± 0.9 (N = 12)	↓1.1 ± 0.5 (N = 11)	↑1.3 ± 1.0 (N = 12)	**↓0.5** ± 0.5 (N = 12)**	0.8 ± 1.0 (N = 10)
Week 27	1.1 ± 0.8 (N = 12)	0.6 ± 0.5 (N = 12)	↓0.7 ± 0.5 (N = 11)	0.6 ± 0.7 (N = 12)	↓0.8 ± 0.8 (N = 12)	↑0.9 ± 0.5 (N = 12)	**↓0.4* ± 0.5 (N = 12)**	↑0.9 ± 0.9 (N = 10)
Week 53	3.4 ± 2.1 (N = 23)	2.0 ± 1.2 (N = 23)	↑3.9 ± 2.5 (N = 23)	↓1.8 ± 1.5 (N = 23)	**↓2.6* ± 1.6 (N = 24)**	↓1.9 ± 1.1 (N = 24)	↓2.7 ± 2.0 (N = 23)	↓1.6 ± 1.0 (N = 20)
Plasma alanine aminotransferase (IU/L)								
Week 14	84.3 ± 12.0 (N = 12)	66.2 ± 16.9 (N = 12)	↑92.8 ± 13.6 (N = 11)	↑79.3 ± 38.9 (N = 12)	**↑110.9** ± 10.1 (N = 11)**	**↑88.2** ± 16.5 (N = 12)**	**↑109.6** ± 23.1 (N = 12)**	**↑90.5** ± 20.2 (N = 10)**
Week 27	98.3 ± 14.6 (N = 12)	87.3 ± 33.3 (N = 12)	↓94.1 ± 25.9 (N = 12)	↓83.8 ± 19.3 (N = 12)	↑100.8 ± 29.8 (N = 12)	↑87.4 ± 27.6 (N = 12)	↑110.5 ± 18.6 (N = 23)	↑93.4 ± 18.3 (N = 10)
Week 53	87.3 ± 49.4 (N = 23)	74.8 ± 42.6 (N = 24)	↑89.0 ± 36.2 (N = 23)	↑89.7 ± 46.5 (N = 23)	↑91.8 ± 26.3 (N = 24)	**↑104.6* ± 54.1 (N = 24)**	↑102.1 ± 49.2 (N = 23)	↑80.1 ± 44.8 (N = 20)
Plasma aspartate aminotransferase (IU/L)								
Week 14	97.5 ± 17.8 (N = 12)	90.4 ± 18.4 (N = 12)	↓92.4 ± 15.2 (N = 11)	↑103.4 ± 47.4 (N = 12)	↓91.6 ± 7.6 (N = 11)	↓89.4 ± 13.8 (N = 12)	↑101.9 ± 17.7 (N = 12)	↑92.7 ± 10.0 (N = 10)
Week 27	120.0 ± 29.7 (N = 12)	157.1 ± 85.3 (N = 12)	↓105.3 ± 27.0 (N = 11)	↓130.4 ± 58.4 (N = 12)	↓108.4 ± 38.0 (N = 12)	**↓116.9* ± 49.1 (N = 12)**	↓113.3 ± 22.3 (N = 12)	↓132.8 ± 37.4 (N = 10)
Week 53	122.1 ± 57.8 (N = 23)	133.9 ± 64.2 (N = 24)	↑123.0 ± 47.5 (N = 23)	**↑178.7* ± 99.4 (N = 23)**	↓118.3 ± 28.9 (N = 24)	**↑198.3** ± 103.7 (N = 24)**	↑132.0 ± 86.3 (N = 23)	↑138.3 ± 86.7 (N = 20)
Plasma creatine kinase (IU/L)								
Week 14	118.2 ± 14.9 (N = 12)	96.7 ± 16.5 (N = 12)	↑123.5 ± 18.9 (N = 11)	↑107.5 ± 24.6 (N = 12)	↑127.3 ± 21.5 (N = 11)	↑107.3 ± 15.4 (N = 12)	**↑143.7** ± 32.9 (N = 12)**	**↑124.1** ± 19.0 (N = 10)**
Week 27	96.6 ± 25.7 (N = 12)	77.3 ± 17.7 (N = 12)	↓89.1 ± 8.3 (N = 11)	↑79.7 ± 13.6 (N = 12)	↑103.4 ± 35.4 (N = 12)	↑85.4 ± 16.0 (N = 12)	↓93.1 ± 13.7 (N = 12)	**↑107.9** ± 20.9 (N = 10)**
Week 53	118.3 ± 40.8 (N = 23)	101.3 ± 27.6 (N = 24)	↓116.7 ± 23.2 (N = 23)	↑117.5 ± 48.8 (N = 23)	↑138.2 ± 50.5 (N = 24)	**↑281.6* ± 828.1 (N = 24)**	↑133.1 ± 33.2 (N = 23)	↑139.4 ± 101.7 (N = 20)
Plasma sodium (mmol/L)								
Week 14	142.5 ± 1.9 (N = 12)	142.4 ± 1.7 (N = 12)	↓142.1 ± 0.8 (N = 11)	↑143.4 ± 1.8 (N = 12)	↓142.2 ± 1.7 (N = 11)	↑143.0 ± 1.5 (N = 12)	**↑143.8* ± 1.6 (N = 12)**	↓142.1 ± 1.1 (N = 10)
Week 27	142.6 ± 0.9	142.3 ± 1.5	↓141.9 ± 1.2	↓142.2 ± 1.0	142.6 ± 1.4	↓142.2 ± 1.5	142.6 ± 0.7	↓142.2 ± 1.3

Table B.6.5.6-7 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Clinical chemistry findings

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	(N = 12)	(N = 12)	(N = 11)	(N = 12)	(N = 12)	(N = 12)	(N = 12)	(N = 10)
Week 53	146.0 ± 1.9 (N = 23)	143.8 ± 1.6 (N = 22)	↑146.1 ± 2.3 (N = 23)	**↑145.0** ± 2.1 (N = 22)**	↑146.3 ± 1.5 (N = 12)	↑144.0 ± 1.9 (N = 22)	↓145.6 ± 2.2 (N = 22)	↑144.4 ± 2.2 (N = 17)
Plasma chloride (mmol/L)								
Week 14	101.3 ± 1.9 (N = 12)	103.7 ± 1.1 (N = 12)	↑101.8 ± 1.8 (N = 11)	↑104.4 ± 1.3 (N = 12)	↑101.8 ± 1.3 (N = 11)	↑103.9 ± 0.9 (N = 12)	↑101.9 ± 1.1 (N = 12)	↓102.8 ± 1.1 (N = 10)
Week 27	101.2 ± 1.5 (N = 12)	102.5 ± 1.8 (N = 12)	↑101.6 ± 1.0 (N = 11)	↑102.7 ± 1.7 (N = 12)	↓101.0 ± 2.0 (N = 23)	↑102.9 ± 1.6 (N = 12)	↑101.3 ± 1.2 (N = 12)	↓102.3 ± 1.3 (N = 10)
Week 53	106.2 ± 2.7 (N = 23)	104.6 ± 2.6 (N = 22)	↓105.9 ± 2.3 (N = 23)	**↑105.4** ± 2.9 (N = 22)**	↓105.5 ± 2.0 (N = 23)	**↑105.4* ± 2.4** (N = 22)	↓105.5 ± 2.1 (N = 22)	↓104.3 ± 2.4 (N = 17)
Plasma calcium (mmol/L)								
Week 14	2.88 ± 0.04 (N = 12)	2.79 ± 0.06 (N = 12)	↓2.84 ± 0.04 (N = 11)	↓2.78 ± 0.05 (N = 12)	**↓2.82** ± 0.05 (N = 11)**	2.79 ± 0.05 (N = 12)	↓2.86 ± 0.04 (N = 12)	**↑2.75* ± 0.04 (N = 10)**
Week 27	2.82 ± 0.07 (N = 12)	2.81 ± 0.06 (N = 12)	**↓2.78* ± 0.05 (N = 11)**	**↓2.75** ± 0.04 (N = 12)**	↓2.78 ± 0.04 (N = 12)	**↓2.76* ± 0.07 (N = 12)**	↓2.80 ± 0.05 (N = 12)	**↓2.74** ± 0.07 (N = 10)**
Week 53	2.86 ± 0.10 (N = 23)	2.99 ± 0.16 (N = 23)	↑2.90 ± 0.11 (N = 23)	↓2.94 ± 0.14 (N = 23)	**↑2.94** ± 0.14 (N = 24)**	**↓2.88** ± 0.13 (N = 24)**	↓2.85 ± 0.09 (N = 23)	↓2.92 ± 0.16 (N = 20)
Plasma phosphorus (mmol/L)								
Week 14	1.96 ± 0.17 (N = 12)	1.33 ± 0.18 (N = 12)	↓1.88 ± 0.26 (N = 11)	↑1.59** ± 0.24 (N = 12)	↓1.82 ± 0.26 (N = 11)	↑1.48 ± 0.31 (N = 12)	↑2.09 ± 0.26 (N = 12)	**↑1.74** ± 0.21 (N = 10)**
Week 27	1.48 ± 0.16 (N = 12)	1.29 ± 0.13 (N = 12)	↑1.50 ± 0.11 (N = 11)	↓1.18 ± 0.20 (N = 12)	↑1.55 ± 0.10 (N = 12)	↓1.15* ± 0.28 (N = 12)	↑1.73** ± 0.21 (N = 12)	**↑1.39* ± 0.20 (N = 10)**
Week 53	1.37 ± 0.36 (N = 23)	1.94 ± 0.42 (N = 24)	↑1.49 ± 0.36 (N = 23)	↑1.97 ± 0.59 (N = 23)	**↑1.64** ± 0.39 (N = 24)**	**↓1.62** ± 0.26 (N = 24)**	**↑1.56* ± 0.26 (N = 23)**	↓1.82 ± 0.26 (N = 20)

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

G. URINALYSIS

Urinary volume was decreased in mid and high dose males at week 13 and 26. At week 26 low dose males also showed reduced urine volume. Without any concomitant findings the toxicological relevance of this change is considered questionable.

Table B.6.5.6-8 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Urine clinical chemistry

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine volume [mL]								
Week 13	8.75 ± 1.91	4.71 ± 1.54	↓7.55 ± 2.25	↓4.38 ± 1.52	**↓6.83** ± 1.63**	↓3.71 ± 1.36	**↓5.63** ± 1.72**	↓3.95 ± 1.50

Table B.6.5.6-8 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Urine clinical chemistry

	Dose group (ppm)							
	0		2000		8000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
	(N = 12)	(N = 12)	(N = 11)	(N = 12)	**(N = 12)**	(N = 12)	**(N = 12)**	(N = 10)
Week 26	9.50 ± 2.27 (N = 12)	4.04 ± 1.59 (N = 12)	↓**7.36**** **± 1.96** **(N = 12)**	↑4.21 ± 1.45 (N = 12)	↓**6.63**** **± 1.46** **(N = 12)**	↓3.38 ± 1.55 (N = 12)	↓**6.21**** **± 1.75** **(N = 12)**	↓3.65 ± 1.33 (N = 10)
Week 52	7.88 ± 2.06 (N = 12)	5.29 ± 1.70 (N = 12)	↓7.79 ± 2.78 (N = 12)	↓4.54 ± 1.81 (N = 12)	↓6.58 ± 1.59 (N = 12)	↓4.25 ± 1.41 (N = 12)	↓**5.88* ± 1.86** **(N = 12)**	↓4.05 ± 1.48 (N = 10)
Urine specific gravity								
Week 13	1.038 ± 0.006 (N = 12)	1.047 ± 0.014 (N = 12)	↑1.041 ± 0.009 (N = 11)	↑1.048 ± 0.013 (N = 12)	↑1.041 ± 0.005 (N = 12)	↑1.048 ± 0.012 (N = 12)	↑**1.049* ± 0.011** **(N = 12)**	↑1.049 ± 0.009 (N = 10)
Week 26	1.038 ± 0.005 (N = 12)	1.053 ± 0.017 (N = 12)	↑1.042 ± 0.009 (N = 11)	↓1.048 ± 0.010 (N = 12)	↑1.046 ± 0.010 (N = 12)	↓1.051 ± 0.013 (N 0 12)	↑1.043 ± 0.008 (N = 12)	↓1.049 ± 0.004 (N = 10)
Week 52	1.044 ± 0.006 (N = 12)	1.044 ± 0.009 (N = 12)	↓1.041 ± 0.006 (N = 12)	↑1.051 ± 0.015 (N = 12)	↑1.048 ± 0.012 (N = 12)	↑1.047 ± 0.009 (N = 12)	↑1.049 ± 0.009 (N = 12)	↑1.047 ± 0.009 (N = 10)
Urine [pH]								
Week 13	6.42 ± 0.51 (N = 12)	5.75 ± 0.45 (N = 12)	↓6.36 ± 0.50 (N = 12)	↑5.92 ± 0.29 (N = 12)	↓6.33 ± 0.49 (N = 12)	↑5.92 ± 0.67 (N = 12)	↓**6.00* ± 0.00** **(N = 12)**	↓5.40 ± 0.52 (N = 10)
Week 26	6.08 ± 0.29 (N = 12)	5.08 ± 0.29 (N = 12)	↑6.18 ± 0.40 (N = 11)	↑**5.50* ± 0.52** **(N = 12)**	6.08 ± 0.29 (N = 12)	↑**5.67**** **± 0.49** **(N = 12)**	↓5.92 ± 0.29 (N = 12)	↑5.30 ± 0.48 (N = 10)
Week 52	6.00 ± 0.00 (N = 12)	5.67 ± 0.49 (N = 12)	↑6.33 ± 0.49 (N = 12)	↓5.58 ± 0.51 (N = 12)	↑6.08 ± 0.67 (N = 12)	5.67 ± 0.49 (N = 12)	↓5.75 ± 0.62 (N = 12)	↓5.40 ± 0.52 (N = 10)

* Statistically significant from control ($p \leq 0.05$; Student's t-test, two-sided)
** Statistically significant from control ($p \leq 0.01$; Student's t-test, two-sided)

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic effects.

Organ weights

There were no treatment- and dose-related effects on organ weights when corrected for bodyweight.

Histopathology

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 glyphosate acid. This effect was also evident in females receiving 8000 ppm. This change was considered to be related to treatment and consequently the salivary glands of the 8000 ppm dose group were examined. The examples of focal parotid basophilia seen at this dose showed an increase in incidence in females (2 in control versus 6 at 8000 ppm).

Prostatitis was observed in 9 high dose males compared to 2 in the control animals. In addition, an increase in the incidence proliferative cholangitis of the liver was observed in high dose females.

No treatment-related neoplasms were found. However, it should be noted that a 1-year study is not adequate to address carcinogenicity.

Table B.6.5.6-9 Glyphosate Acid: One Year Dietary Toxicity Study in Rats ([redacted] 1996): Non-neoplastic findings (terminal)

Effect		Dose group (ppm) 0 ♂	0 ♀	2000 ♂	2000 ♀	8000 ♂	8000 ♀	20000 ♂	20000 ♀
Liver									
Proliferative cholangitis	Total	12	10	0	0	0	0	11	19
	Minimal	12	10	0	0	0	0	10	18
	Slight	0	0	0	0	0	0	1	1
Prostate gland									
Prostatitis	Total	2	-	0	-	0	-	9	-
	Minimal	1	-	0	-	0	-	4	-
	Slight	0	-	0	-	0	-	4	-
	Marked	1	-	0	-	0	-	1	-
Salivary glands									
Basophilia of parotid acinar cells	Total	2	2	0	0	3	6	13	15
	Minimal	2	2	0	0	3	6	10	8
	Slight	0	0	0	0	0	0	3	5
	Moderate	0	0	0	0	0	0	0	2

Assessment and conclusion by applicant:
Based on body weight and salivary gland effects at 20000 ppm, the NOAEL for toxicity for glyphosate acid was 8000 ppm equivalent to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females. There was no evidence of carcinogenicity. At the mid dose, effects on the acinar cells of the parotid salivary gland were slightly increased (minimal severity) in females only. These effects when occurring at a minimal severity are generally considered adaptive being due to pH changes of the glyphosate acid. The body weight reduction was neither dose nor time-dependent. The effects on body weight was furthermore rather low at the mid dose level (<10 %) at most time points. The higher alkaline phosphatase activity at 8000 and 20000 ppm is not considered an adverse effect as no other enzyme activities were affected indicative of organ damage. Furthermore, histopathology did not indicate any damage of organs, e.g. liver. No indication of neoplastic changes was observed.

Assessment and conclusion by RMS:

For the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the case there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach.

Histopathology revealed an increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland in both sexes at 20000 ppm (1409 mg/kg bw/day). Incidence: 57% (males) and 75% (females). Severity grade: minimal to slight (males) and minimal to moderate (females).

At 8000 ppm (560 mg/kg bw/day) focal basophilia of parotid acinar cells were all of minimal severity and the incidence for males (12.5%) was comparable to that in the control group (8.7%), while the incidence for females (25%) was above the control group (8.3%). No statistical analyses were conducted and no historical control data are available. Since the salivary gland weights were not investigated in the study it is proposed to set the LOAEL at 8000 ppm based on the effect in females as a precautionary approach although the severity grade of findings observed at this dose level was minimal.

At 2000 ppm no effects on parotid acinar cells were observed. Thus, the **NOAEL is set at 2000 ppm** (equal to 167 mg/kg bw/day in females).

This NOAEL is in line with the NOAEL set during the previous evaluation (RAR 2015).

B.6.5.7. Long-term toxicity – rat, study 7

Data point:	CA 5.5/007 CA 5.5/008 CA 5.5/009
Report author	[redacted].
Report year	1993
Report title	Glyphosate – 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks)
Report No	7867
Document No	153-GLY
Guidelines followed in study	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general compliance with OECD 453
Deviations from current test guideline (OECD 453, 2018)	The following deviations were noted from the current OECD test guideline : - haematological examination of prothrombin time and activated partial thromboplastin time (APTT) were not performed; - urinalysis was performed without determining glucose; - organ weight of the thyroid/parathyroid was not determined; - histopathological examinations were performed without Harderian gland, cervix, coagulating glands, lacrimal glands, seminal vesicles and vagina.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant: Valid, Category 2a Conclusion AGG: Although some deviations were noted compared to the current OECD test guideline these are not considered to affect the reliability of the parameters that were examined and therefore the study is considered to be acceptable.

Full summary

The chronic toxicity and carcinogenic potential of glyphosate technical was assessed in a 104-week feeding study in male and female Sprague-Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day glyphosate technical for 24 months. In addition, five groups of 35 rats/sex, receiving daily dietary doses of, 0, 10, 100, 300 or 1000 mg/kg bw/day, were included for interim sacrifice at the 12th month for evaluation of chronic toxicity. Observations covered clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry and urinalysis, as well as organ weights, necropsy and histopathological examination.

Achieved doses throughout the study period were generally close to nominal. There were no treatment-related deaths or clinical signs in any of the dose-groups. Ophthalmoscopic examinations showed no inter-group differences. At 1000 mg/kg bw/day males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights. Food and water consumption did not differ significantly from the controls. Moreover, there were no treatment-related changes in haematological parameters. Clinical chemistry evaluation indicated a treatment-related increase of ALP in males of the 1000 mg/kg bw/day dose group and females of the 300 and 1000 mg/kg bw/day dose groups, as well as reduced urinary pH in males at 1000 mg/kg bw/day.

At necropsy no treatment-related gross lesions were observed. Organ weight data showed reduced relative liver weights in females at 100, 300 and 1000 mg/kg bw/day at interim kill in week 52, but not after 104 weeks. At week 52 salivary gland weights were increased in 100, 300 and 1000 mg/kg bw/day dose group males. Combined sublingual and submaxillary gland weights were also increased in males and females treated with 1000 mg/kg bw/day. However there were no significant inter-group differences by week 104. Histopathological examination noted cellular alteration of in submaxillary and parotid salivary glands in males and females of the 300 and 1000 mg/kg bw/day dose groups (week 52) and the 100, 300 and 1000 mg/kg bw/day dose groups of both sexes at week 104. These changes followed a dose-related pattern and are considered treatment related. Considering that the increase at 300 mg/kg bw/day and higher was both in total incidence as well as severity and coincided with the increase in salivary gland weight the observed effect was considered to be treatment related and adverse.

No treatment-related neoplastic lesions were observed at termination.

The NOAEL was concluded to be 100 mg/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	229-JaK-5-1; 229-Jak-142-6
Purity:	98.9%; 98.7%
Stability of test compound:	At least two years at ambient temperature in the dark
2. Vehicle and/or positive control:	Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks upon arrival at testing facility
Sex:	Males and females
Weight at dosing:	Males: 208 ± 1.9 g, females: 148.5 ± 1.6 g
Acclimation period:	14 days
Diet/Food:	SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of five per sex in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 - 20 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1990-02-16 to 1992-03-09

Animal assignment and treatment:

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day glyphosate technical. An additional five groups with 35 rats per sex receiving daily dietary doses of 0, 10, 100, 300 or 1000 mg/kg bw/day were included for the toxicity study. Fifteen rats per sex and per dose of the toxicity study were scheduled for interim sacrifice after 12 months. The dose levels were selected based on the results of a 13-week dietary toxicity study in rats.

Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes.

Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets at approximately fortnight intervals for the first 12 weeks and in intervals of 2 month thereafter.

The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.

Clinical observations
A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal. An ophthalmoscopic examination was conducted on 20 rats per sex of each group of the oncogenicity study before the start of the study and on 20 rats per sex of the control and high-dose group of the oncogenicity study at weeks 24 and 50. In addition, an ophthalmoscopic examination was conducted on all control and high-dose rats of the oncogenicity and toxicity study at week 102.

Body weight
Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.

Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology and clinical chemistry
Individual blood samples for haematology and clinical chemistry evaluations were collected from the orbital sinus of 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Individual blood smears for differential blood counts were taken from the tail vein after approximately 52, 78, and 103 weeks of dosing from all surviving animals of the oncogenicity study.

Haematology
The following parameters were measured: Haemoglobin, haematocrit, total erythrocyte count total leukocyte count, differential leukocyte count, MCH, MCV, MCHC, platelets, and clotting time. Absolute indices were calculated.
Differential blood counts were evaluated with blood smear samples from all control and high-dose animals of the oncogenicity study at weeks 53 and 79. In addition, differential blood cell counts were evaluated on all surviving animals of the oncogenicity study at week 104.

Blood chemistry
The following parameters were measured: Total proteins, albumin, albumin-globulin ratio, ALT, AST, ALP, blood urea nitrogen, blood glucose, sodium, potassium, chloride, cholesterol, creatinine, calcium, phosphate, total bilirubin, plasma cholinesterase, creatine phosphokinase and red blood cell cholinesterase.

Brain cholinesterase activity determination
Brain cholinesterase activity was determined from 10 rats per sex from each dose group at the week 52 and 104 necropsies. Approximately 0.5 g of brain was removed at the week 52 and 104 necropsies and stored at -20 °C until analysis.

Urinalysis
Individual urine samples were collected from 10 rats/sex of each study group of the toxicity study after approximately 14, 25, 51, 78 and 102 weeks. Samples were taken where possible, on the same animals at each time point. Samples were collected over a period of 4 hours of food and water deprivation in metabolism cages. The following measurements were made: volume, specific gravity, pH, urobilinogen, bilirubin, blood pigments, protein, glucose, ketones, microscopy of sediments.

Sacrifice and pathology
At interim kill after 52 weeks 15 rats per sex from each toxicity study group were sacrificed and necropsied. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All pre-terminally dead and moribund sacrificed rats were also necropsied.

The following organs were weighed from all interim kill animals of the toxicity study and from 10 rats per sex per group of the oncogenicity study: adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), parotid salivary glands, pituitary, prostrate, sublingual and submaxillary salivary glands (weighed together), spleen, testes including epididymides, thymus and uterus.

The following organs were collected: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs, mammary gland, lymph nodes (mesenteric and submandibular), muscle (thigh), nasal cavity (oncogenicity study only), oesophagus, optic nerve, ovaries (with fallopian tubes), pancreas, parotid salivary glands, pituitary, prostrate, sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), sublingual salivary glands, submaxillary salivary glands, testes with epididymides, thymus, thyroid/parathyroid, tongue, trachea and uterus.

A detailed histopathological examination was performed on all tissues collected from the control and high-dose animals at interim kill, all oncogenicity study animals, and all animals that died or were killed in extremis. In addition, a histopathological examination of the liver, kidneys and lungs was performed on all other toxicity study animals at interim kill and all oncogenicity study animals. Furthermore, the salivary glands of all low- and mid-dose animals at interim kill and the oncogenicity study were examined.

Statistics
Haematology, clinical chemistry, organ weight and body weight data were analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANOVA was used and pair wise comparisons made *via* Student's t-test using Fisher´s F-protected LSD. If the variances were heterogeneous log or square root, transformations were used. If the variances remained heterogeneous a non-parametric test (e.g. Kruskal-Wallis ANOVA) was used. Organ weights were also analysed conditional on body weight (i.e. ANOVA). Differences in survival between the control and test substance groups from the oncogenicity study were assessed graphically using Kaplan-Meier plots and tested formally using the Gehan-Wilcoxon test. Because no notable survival differences were evident, histological lesion incidences were analysed using Fisher Exact test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10 %).

B. MORTALITY
There were 336 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment-related. There were also no significant treatment-related effects on the survival times in the oncogenicity study.

The numbers of pre-terminal deaths are summarised in the table below.

Table B.6.5.7-1 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] et al., 1993): Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Sex	Dose group (mg/kg bw/day)*				
	0	10	100	300	1000
Male	27/85	32/85	25/85	26/85	26/85
Female	42/85	41/85	42/85	40/85	35/85

* Number of dead / total number

C. CLINICAL OBSERVATIONS

The only notable clinical sign was pale faeces, from weeks 16-104, the majority or all the cages of animals (males and females) in the 300 and 1000 mg/kg /day dose groups had pale faeces. However, this clinical sign was not considered to be toxicologically significant. There were no other notable clinical signs considered to be treatment related.

Ophthalmoscopy examinations demonstrated no inter-group differences.

D. BODY WEIGHT

The high-dose group males and females had statistically significant reductions in body weight throughout the study. Reductions started at week one of dosing and were still apparent at week 104. The high-dose group males displayed the greatest reduction in body weights and body weight gains. In the 100 mg/kg bw/day toxicity groups there was a >10% decrease in body weight gain males at week 0-52. However, the effect was not dose-related and a similar effect was not observed in the oncogenicity group. Therefore, this effect was not considered to be a treatment related adverse effect. The mean body weight gain data are summarised in the table below.

Table B.6.5.7-2 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] *et al.*, 1993): Body weight development (mean values) after 52 and 104-week dietary exposure to glyphosate technical – oncogenicity study

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Oncogenicity groups (n=50)										
Weight gain (g) (0-52 weeks)	514	265	498	285	523	270	500	274	450	243
% of control	--	--	97	108	102	102	97	103	88	92
Weight gain (g) 0-104 weeks	635	376	609	445	644	391	623	405	549	333
% of control	--	--	96	118	101	104	98	108	86	89
Toxicity groups (n=35)										
Weight gain (g) (0-52 weeks)	516	254	477	267	454	276	456	278	404	244
% of control	-	-	92	105	88	109	88	109	78	96
Weight gain (g) 0-104 weeks	634	374	523	397	605	389	598	349	430	310
% of control	-	-	82	106	95	104	94	93	68	83

E. FOOD AND WATER CONSUMPTION AND COMOUND INTAKE

There were no treatment-related effects on food and water consumption for either sex noted during the study. The overall group mean achieved doses are summarised in the table below.

Table B.6.5.7-3 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] *et al.*, 1993): Group mean achieved dose levels – oncogenicity study

Dose group	Nominal dose (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)		Mean achieved dose level (% of nominal)	
		Males	Females	Males	Females
Oncogenicity groups (n=50)					
1 (control)	0	--	--	--	--
2 (low)	10	10	10	100	100
3 (mid I)	100	101	103	101	103
4 (mid II)	300	306	311	102	104
5 (high)	1000	1007	1018	101	102
Toxicity groups (n=35)					
1 (control)	0	--	--	--	--
2 (low)	10	11	12	110	120
3 (mid I)	100	112	109	112	109
4 (mid II)	300	320	347	107	116
5 (high)	1000	1147	1134	115	113

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Haemoglobin, haematocrit and mean corpuscular haemoglobin were occasionally increased in 100 and 1000 mg/kg bw/day dose group males. Haemoglobin was also increased in males from the 300 mg/kg bw/day dose group and females from the 1000 mg/kg bw/day group. Females of the 1000 mg/kg bw/day dose group also had increased levels of mean corpuscular haemoglobin.

The haematological changes were not considered to be treatment related due to the lack of a clear dose–response relationship. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In the absence of any histopathological change these small increases are not considered to be of toxicological significance (see table below).

Table B.6.5.7-4 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] et al., 1993): Haematology findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Haemoglobin (g/dL)										
Week 14/15	15.6	15.5	↑15.8	↓**15.0***	↑16.2	↓**15.0***	↑16.2	15.5	↑16.2	↑15.9
Week 25/26	15.3	15.2	↑15.5	↓14.9	↑**16.1****	↓14.9	↑**15.9***	↑15.4	↑**16.4****	↑15.6
Week 51/52	15.3	14.7	↑15.5	↓14.6	↑15.9	↓14.5	↑15.4	14.7	↑15.6	↑**15.3***
Week 78/79	15.1	14.1	↓14.3	↓13.8	↑15.7	↑14.4	↓14.6	↑14.4	↑15.4	↑15.1
Week 102/103	14.0	12.1	↓13.1	↑13.6	↑14.3	↑13.1	↓13.8	↑13.3	↑14.6	↑12.9
Haematocrit (L/L)										
Week 14/15	0.397	0.396	↑0.405	↓0.386	↑0.406	↓0.387	↑0.407	↓0.395	↑0.411	↑0.407

Table B.6.5.7-4 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] et al., 1993): Haematology findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Week 25/26	0.388	0.392	↑0.389	↓0.389	↑**0.409****	↓0.384	↑0.399	↑0.398	↑**0.409****	↑0.403
Week 51/52	0.406	0.394	↑0.415	↓0.388	↑0.415	↓0.386	↑0.410	↓0.392	↑0.414	↑0.408
Week 78/79	0.405	0.382	↓0.386	↓0.375	↑0.415	0.382	↓0.392	↑0.387	↑0.411	↑0.406
Week 102/103	0.392	0.343	↓0.365	↑0.381	↑0.394	↑0.367	↓0.387	↑0.369	↑0.401	↑0.363
MCH (pg)										
Week 14/15	21.3	22.6	↓21.1	↓22.5	↑21.7	↓22.4	↑21.9	↓22.4	↑21.8	↑22.8
Week 25/26	21.2	22.4	↑21.4	22.4	↑21.9	22.4	↑21.9	↓22.2	↑22.0	↑22.8
Week 51/52	20.2	22.1	↓20.1	↑22.3	↑**21.1***	22.1	↑20.8	↑22.2	↑**20.9***	↑22.7
Week 78/79	20.1	22.3	↓19.7	↑22.4	↑**20.8***	↑22.4	↑20.6	↑23.0	↑**20.9***	↑**23.1****
Week 102/103	20.4	22.3	↓20.1	22.3	↑20.1	↓22.0	↑20.9	↑22.6	↑20.6	↑22.7
WBC (x 10-9/L)										
Week 14/15	14.0	12.0	↑14.5	↑13.3	↓13.4	12.0	↓13.7	↓11.1	↑14.2	↓12.0
Week 25/26	13.4	8.8	↓13.2	↑10.3	↓11.8	↑9.9	↓12.2	↑8.9	↓12.7	↑10.5
Week 51/52	12.8	7.9	↑13.7	↑9.1	↓11.7	↓7.7	↑12.9	↓7.4	↓12.4	↑8.8
Week 78/79	12.4	7.7	↑13.6	↓7.3	↓10.9	↑8.1	↑13.6	↓6.8	↓10.6	↓7.0
Week 102/103	10.5	10.1	↑12.2	↓**7.1***	↓10.3	↓**6.4****	↑11.6	↓**7.3***	↓9.5	↓8.4
Lymphocytes (x 10-9/L)										
Week 14/15	11.7	10.8	↑12.6	↑11.9	↑12.0	↑10.9	↑11.8	↓9.2	↑12.2	↓10.7
Week 25/26	10.7	7.1	↑10.8	↑8.2	↓9.6	↑8.1	↓10.1	↑7.4	↓10.3	↑8.6
Week 51/52	10.9	6.5	↑11.0	↑7.4	↓9.7	↑6.6	↓10.8	↓6.0	↓10.3	↑7.5
Week 78/79	10.0	5.7	↑10.3	↓5.6	↓8.7	↑6.4	↑10.1	↓4.8	↓8.5	↓5.6
Week 102/103	7.6	5.7	↑8.0	↓4.8	↓7.3	↓**4.3****	↑7.8	↓**4.7***	↓6.7	↓5.6

*: $p < 0.05$
**: $p < 0.01$
***: $p < 0.001$

Clinical chemistry

Clinical chemistry measurements showed significant increased alkaline phosphatase levels in males at 1000 mg/kg bw/day and in females at 100, 300 and 1000 mg/kg bw/day. Although the increases were of small magnitude they were consistent and might be treatment-related. Since the effect in the 300 and 1000 mg/kg bw/day dose group

was fairly consistent throughout the study and was of quite a high magnitude (up to 67% at 300 mg/kg bw/day and up to 72% at 1000 mg/kg bw/day and considering

No effect on brain cholinesterase activity occurred at 52 or 104 weeks.

Table B.6.5.7-5 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] et al., 1993): Clinical chemistry findings (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
ALP (IU/L)										
Week 14	287	182	↑329	↓158	↑320	↑213	↑334	↑223	↑**461*** **	↑**244***
Week 25	251	148	↑272	↑152	↑267	↑**201***	↑306	↑**227****	↑**367****	↑**225****
Week 51	308	144	↓293	↓143	↑310	↑**190***	↑353	↑**195***	↑403	↑**221****
Week 78	258	124	↑286	↑139	↑284	↑172	↑**351*** **(+36%)**	↑**207**** **(+67%)**	↑**414*** **	↑**186***
Week 102	212	190	↑265	↓161	↑**287***	↑193	↑267	↑228	↑**365*** ** **(+72%)**	↑**286*** **(+51%)**

*: $p < 0.05$
**; $p < 0.01$
***: $p < 0.001$

G. URINALYSIS

Urinary pH was slightly reduced in males at 1000 mg/kg bw/day. This change was consistent with that found in a previously conducted 13-week toxicity study with glyphosate.

H. NECROPSY

Gross pathology

There were no treatment-related macroscopic findings observed at the interim and terminal kill necropsies.

Organ weights

At the interim kill (week 52) absolute liver weights were reduced in males and females at doses of 100 mg/kg bw/day and above. However, no effect on relative liver weight was observed in males. However, a decrease was observed in females at week 52.
Absolute adrenal weights were reduced in males at 300 and 1000 mg/kg bw/day. However, this finding was also not confirmed by the sensitive means of covariance analysis, i.e. with correction for final body weight.

At the terminal kill (week 104) no statistical significant decrease in liver and adrenal weights was noted in any dose group. Absolute kidney weight was reduced in males at 100 and 1000 mg/kg bw/day after 104 weeks, but a clear dose relationship was lacking.

At 52 weeks parotid salivary gland weight was increased in males at 100, 300 and 1000 mg/kg bw/day. Combined sublingual and submaxillary gland weight was increased in high-dose males and females. However, salivary gland weights were not affected at week 104 at any dose level.

Table B.6.5.7-6 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] et al., 1993): Organ weights (group mean values)

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver weight										

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Absolute week 52	24.63	14.46	22.80	13.17	**21.58***	13.01	**20.70** (-16%)**	14.18	**19.70** (-20%)**	**12.04** (-17%)**
Absolute, week 104	29.92	16.38	23.21	18.53	22.07	18.30	24.67	18.09	23.38	15.13
Relative week 52	22.40	14.60	21.62	13.89	21.70	**13.15***	21.81	**12.87** (-12%)**	21.88	**12.34*** (-15%)**
Relative week 104	24.51	17.21	23.49	17.72	21.86	18.74	23.76	16.97	23.62	15.91
Kidney weight										
Absolute week 52	4.61	2.85	4.31	2.87	4.21	2.79	4.21	3.07	4.15	2.78
Absolute, week 104	5.96	3.19	5.69	3.34	**4.61****	3.27	5.52	3.42	**4.82***	3.07
Relative week 52	4.32	2.87	4.16	2.96	4.23	2.80	4.36	2.91	4.44	2.81
Relative week 104	5.94	3.26	5.68	3.27	**4.61****	3.31	5.54	3.32	**4.81****	3.14
Parotid salivary glands										
Absolute week 52	0.18	0.25	0.22	0.28	**0.28***	0.26	**0.32** (+77%)**	0.26	**0.38*** (+111%)**	0.29
Absolute, week 104	0.30	0.21	0.23	0.21	0.28	0.21	0.26	0.33	0.31	0.28
Relative week 52	0.17	0.25	0.22	0.29	**0.28***	0.26	**0.33*** (+94%)**	0.24	**0.39*** (+129%)**	0.29
Relative week 104	0.30	0.21	0.23	0.21	0.28	0.21	0.26	0.33	0.31	0.28
Sublingual and submaxillary salivary glands										
Absolute week 52	0.88	0.58	0.84	0.62	0.84	0.61	0.84	0.63	**0.99** (+12%)**	**0.67** (+16%)**
Absolute, week 104	0.85	0.60	0.87	0.59	0.84	0.57	0.90	**0.72*** (+20%)**	0.92	0.67
Relative week 52	0.84	0.58	0.82	0.63	0.84	0.61	0.89	0.60	**1.03*** (+23%)**	**0.68*** (+17%)**
Relative week 104	0.86	0.60	0.88	0.59	0.83	0.57	0.88	**0.72*** (+20%)**	0.92	0.66

* p<0.05, **p<0.01, p<0.001

Histopathology

The most notable histological finding was seen in the salivary glands where cellular alteration (recorded when cells were larger and stained deeply basophilic) was seen in submaxillary and parotid salivary glands in males and females at 300 and 1000 mg/kg bw/day at week 52, and in both sexes at 100, 300 and 1000 mg/kg bw/day at week 104. These changes followed a dose-related pattern and are considered to be treatment related; however, these cellular alterations are similar to those seen occasionally in other subchronic or long-term dietary studies and are considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet and are of no adverse consequence. The histological changes in the salivary glands showed no progress to either proliferative or degenerative changes.

Another histopathological finding was a decreased incidence of nephropathy in males at 100, 300 and 1000 mg/kg bw/day at interim kill. This finding was also noted in high-dose males at 104 weeks, but with reduced severity. Nephropathy is a common finding in old rats and as the incidence is decreased this finding is not considered as toxicologically significant.

In addition, the decreased incidence of urothelial hyperplasia in high-dose females at week 52 and 104, as well as in females at 300 mg/kg bw/day at week 104, is also not considered to be of toxicological significance.

Table B.6.5.7-7 Glyphosate – 104 week combined chronic feeding / oncogenicity study in rats with 52 week interim kill (results after 104 weeks) ([redacted] et al., 1993): non-neoplastic findings

	Dose group (mg/kg bw/day)									
	0		10		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Parotid – cellular alteration, oncogenicity group										
Grade +/-	4	1	4	2	8	2	3	2	4	**5**
Grade +	3	0	5	5	9	**9****	**21*****	**9****	**14****	**13*****
Grade ++	0	1	0	1	4	1	**17*****	**9***	**18*****	**18*****
Grade +++	0	0	0	0	0	0	0	1	0	2
Parotid – cellular alteration, toxicity group										
Grade +	0	0	0	0	**9****	2	**8****	**5***	4	**8****
Grade ++	0	0	0	0	1	0	4	3	**7****	**5***
Grade +++	0	0	0	0	0	0	0	0	4	1
Mandibular (submaxillary) – cellular alterations, oncogenicity group										
Grade +/-	7	2	5	0	10	3	14	1	9	6
Grade +	0	9	0	8	**12*****	9	**28*****	15	**22*****	**19***
Grade ++	0	0	0	0	0	0	0	2	0	1
Mandibular (submaxillary) – cellular alterations, toxicity group										
Grade +	0	0	0	-	1	2	**5***	0	**12*****	0

Note: cellular alteration is recorded when cells were larger and stained deeply basophilic

Neoplastic changes

The study report concluded that there was no treatment-related effect on neoplastic findings. However, the RMS noted that there was a slight apparent increase in skin keratoacanthomas in high dose male rats (5/50 compared to 1/50 in controls, 2/25 at 10 ppm, 0/19 at 100 ppm and 0/21 at 300 ppm). Upon re-analysis by an external statistician, a borderline significant trend was observed (P (two-sided) = 0.07 based on the extended Mantel-Haenszel test (stratified Cochran-Armitage trend). However, it should be noted that the tissue skin was not histologically examined in the low and middle dose groups in case of schedules sacrifices but only for animals found death or killed in extremis. Therefore a trend analysis might not be valid. The relevance of this finding in the context to classification for carcinogenicity is discussed in the CLH section in Volume 1.

Assessment and conclusion by applicant:

In conclusion, glyphosate technical was not carcinogenic in male and female Sprague-Dawley rats following continuous dietary exposure of up to 1000 mg/kg bw/day (the limit dose for this type of study) for 104 weeks. Based on the study results and the lack of toxicological significance of the salivary gland findings, as well as a slight and isolated increase of plasma alkaline phosphatase observed at 300 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 300 mg/kg bw/day.

Cellular alteration of the parotid/mandibular salivary gland was considered an adaptive response based on the addition of glyphosate acid to the diet. This effect is in concordance with findings in other studies with glyphosate acid as well as with citric acid (see CA 5.8.2/002). Furthermore, the effect on the salivary glands was not clearly dose related but may be following a threshold effect potentially related to pH changes. This assumption is also supported by the fact that the effects on the salivary glands were already observed after 52-weeks and did not increase in incidence or severity. Increased ALP activity is not considered an adverse effect as the increase was rather low, not accompanied but other liver enzymes and not related to any treatment-related histopathological changes in the liver. Furthermore, the liver weight was comparable between control and treatment groups.

Assessment and conclusion by RMS:

The RMS disagrees with the NOAEL of 300 mg/kg bw/day as proposed by the applicant. At the dose level of 300 mg/kg bw/day and higher considerable increases in ALP were observed (>50%). In addition it is noted that at 300 mg/kg bw/day relative liver weight was decreased in females (-12%). Moreover, significant increases in both incidences and severity of parotic cellular alteration was observed which coincides with the increase in parotid salivary glands weight.

For the interpretation of effects on salivary glands several aspects are considered in order to decide if the effect is adverse or not. The RMS considers salivary gland weight changes and histopathology (severity grade and incidence) along with dose-response and statistical significance. A histopathological finding which is statistically significant is not considered adverse if the severity grade of the finding is minor and there are no salivary gland weight changes. In the case there are no data for salivary gland weights, the effect on histopathology might be considered as potentially adverse in the absence of such data as a precautionary approach.

Histopathology revealed a statistically significant increased incidence of parotid cellular alteration in both sexes at ≥100 mg/kg bw/day. Furthermore, a statistically significant increased incidence of submaxillary cellular alteration was found at ≥100 mg/kg bw/day in males and at ≥300 mg/kg bw/day in females.

At 100 mg/kg bw/day the increased incidence of parotid cellular alteration observed in males was 67% at week 52 (compared to 0% in control) and 43% at week 104 (compared to 14% in control). The severity grade of finding was minimal to moderate. In females, the increased incidence of parotid cellular alteration at week 52 was 13% (compared to 0% in control) and 24% at week 104 (compared to 14% in control). In males, at week 104 the increased incidence of submaxillary cellular alteration was 45% (compared to 14% in control), while no effects on submaxillary gland was observed at week 52 at this dose level. The severity grade of this finding was mild. No historical control data are available. Statistically significant increased parotid gland weights were observed in males (absolute weight: 56%, relative weight: 65%) at the dose level of 100 mg/kg bw/day at week 52 but not at week 104.

At 10 mg/kg bw/day no effects were observed. Thus, the **NOAEL is proposed to be set at 10 mg/kg bw/day** based on adverse effects on salivary glands (histopathological changes and organ weight changes) observed at 100 mg/kg bw/day.

In the previous RAR (2015), a NOAEL of 100 mg/kg bw/day was proposed.

B.6.5.8. Long-term toxicity – rat, study 8

Data point:	CA 5.5/010
Report author	[redacted]
Report year	1990
Report title	Chronic study of glyphosate administered in feed to Albino rats
Report No	[redacted]-10495
Document No	M-651388-02-1
Guidelines followed in study	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982); in general accordance with OECD 453
Deviations from current test guideline (OECD 453, 2018)	The following deviations were noted from the OECD test guideline : - Animals were approximately 8 weeks old at study begin (not >8 weeks); - haematological examinations were performed without prothrombin time and activated partial thromboplastin time; - volume of the urine was not determined; - organ weights of adrenals, heart, ovaries, spleen, thyroid/parathyroid and uterus were not recorded; - histopathological examinations of the coagulating glands, lacrimal glands, and vagina were not performed;
Previous evaluation	Yes, accepted in the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant : Valid, Category 2a

	Conclusion AGG : Although some deviations were noted compared to the current OECD test guideline these are not considered to be critical and therefore the study is concluded to be acceptable.

Full summary

The chronic toxicity and carcinogenic potential of glyphosate was assessed in a 24-month feeding study in 50 male and 50 female Sprague-Dawley rats with 0, 2000, 8000 and 20000 ppm (equal to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females). In addition, 10 rats per sex per dose were included for interim sacrifice after 12 month. Observations covered clinical signs, ophthalmic examinations, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related effects on survival, clinical signs, food consumption, and haematology and clinical chemistry parameters except for an increase in ALP in high dose females. Reduced body weight (gain) was observed in high dose animals as well as increased absolute and relevant liver weight was observed. Increased incidences of inflammation of the stomach mucosa in mid and high dose animals was observed. Pancreatic islet cell adenomas in low-dose males were not dose-related and considered incidental findings. Increased incidences of cataractous lens changes in high-dose males were observed.

An apparent increase in liver cell adenomas was observed in high dose males (8 versus 3 in control).

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White solid
Lot/Batch #:	XLH-264
Purity:	96.5 %
Stability of test compound:	Guaranteed for the study period. Confirmed by analysis.

2. Vehicle and/ or positive control:

Diet

3. Test animals:

Species:	Albino Rat
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	Approx. 8 weeks (at start of study)
Sex:	Males and females
Weight at dosing:	Males: approx. 284 g; females: approx. 221 g
Acclimation period:	29 days
Diet/Food:	Purina Mills certified Rodent Chow #5002 (Purina Mills), *ad libitum*
Water:	Mains drinking water, *ad libitum*
Housing:	In stainless steel cages with wire mesh bottoms suspended over paper bedding

Environmental conditions:	Animal housing & husbandry were in accordance with the provisions of ´Guide to the Care and Use of Laboratory Animal´; USPHS-NIH Publ. No. 85-23 Temperature: 17.8 - 21.1 °C Humidity: 40 - 70 % Air changes: not specified 12 hours light/dark cycle

B: Study design and methods

In life dates: 1987-08-05 to 1989-08-10

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate (equivalent to mean achieved dose levels of 0, 89, 362 and 940 mg/kg bw/day for males and 0, 113, 457 and 1183 mg/kg bw/day for females) for 24 months.

A further ten animals per sex were added to each group and were designated for interim kill after 12 month to study chronic toxicity and non-neoplastic histopathological changes.

Test diets were prepared in approximately weekly intervals by mixing a known amount of the test substance with basal diet. The stability of the dietary formulations were determined by analysis of samples of the low- and high-dose levels after storage at room temperature for 7 and 14 days, and frozen after storage for 35 days. The homogeneity of the test substance in the diet was determined for the low- and high-dose level preparations in the first and 88th week of the study. Analyses for achieved concentrations were done for all dose levels for the first six weeks, and for at least one dose level in weekly intervals thereafter. The stability of the neat test substance was verified by analysis before the start of the study, during month 8, 14 and 21, and after termination.

Clinical observations
All rats were examined for mortality and clinical signs of toxicity twice daily. Detailed clinical observations were conducted weekly. An ophthalmic examination was done in all animals before the start of the study, and prior to termination.

Body weight
Individual body weights were recorded prior to start of treatment, at weekly intervals from Week 1 to 13 and every four weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded at weekly intervals for the first 13 weeks, and every fourth week thereafter.

Haematology and clinical chemistry
Blood was collected from 10 fasted animals per sex and group at Months 6, 12, 18 and at termination. The following parameters were measured:
Haematology : haematocrit, haemoglobin, total erythrocyte count, MCV, MCH, MCHC, platelet count, total leukocyte count, differential leukocyte count, reticulocyte count
Clinical chemistry : alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin, total bilirubin, direct bilirubin, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Month 6, 12, 18 and prior to termination. Sampling was done over a period of about 18-hours *via* metabolism trays. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, urobilinogen and blood. In case that blood and / or protein in excess of the control urine samples were found, the sediment was examined for the presence of bacteria, epithelial cells, erythrocytes, leukocytes, casts or abnormal crystals.

Sacrifice and pathology

A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 12 and 24 month. The following organ weights were determined: brain, kidneys, liver and testes with epididymides.

Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, bone & bone marrow, brain, caecum, colon, duodenum, eyes, gross lesions including palpable masses, Harderian gland, heart, ileum, jejunum, kidneys, liver, lung (with main stem bronchi), lymph nodes (mesenteric and submandibular), muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, sciatic nerve, seminal vesicles, skin (with mammary tissue), spinal cord (cervical, thoracic, lumbar), spleen, stomach, submaxillary salivary gland, testes with epididymides, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix).

Statistics
Dunnett's Multiple Comparison Test (two-tailed) was used for body weights, cumulative body weight changes, food consumption, absolute leukocyte counts, reticulocyte counts, urine pH, urine specific gravity and clinical chemistry data obtained at Months 6, 12 and 18. Fisher´s exact test (one-tailed) was used for incidence of selected ocular lesions, as well as in combination with Bonferroni inequality procedure for incidences of non-neoplastic (at $p \leq 0.01$) and neoplastic lesions (at $p \leq 0.01$ and ≤ 0.05). EHL decision tree analysis was used for evaluation of terminal haematology, clinical chemistry, body weight, absolute and relative organ weight data and organ to brain weight ratios. Depending on the results either parametric (Dunnett´s Test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere´s and / or Mann-Whitney Tests) were applied. Mortality data were analysed by SAS lifetable procedure, and Peto Analysis was used for evaluation of histopathological data.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
The stability analyses proved the neat test substance to be stable throughout the study period.

The stability and homogeneity of glyphosate in diet at concentrations of 2000 and 20000 ppm was satisfactory. The mean achieved concentrations of glyphosate in each dietary preparation were 95% of the nominal concentration.

B. MORTALITY
There were no statistically significant differences in the group survival rates. The percentage of survival in each of the dose groups are summarised below.

Table B.6.5.8-1 Chronic study of glyphosate administered in feed to Albino rats ([redacted] *et al.*, 1990): Percentage survival at termination after 24-month dietary exposure to glyphosate

Sex	Dose group (ppm)			
	0	2000	8000	20000
Male	29	38	34	34
Female	44	44	34	36

C. CLINICAL OBSERVATIONS
There were no treatment-related clinical sings noted except the ophthalmological findings (see below, subsection F).

D. BODY WEIGHT
There were no effects on body weight noted in males of any dose group. In high-dose females body weights were statistically significantly reduced from Week 7 through approximately the 20th month. During this time, absolute body weights gradually decreased to a maximum of 14% below the control value. The maximum difference in body weights was observed at 20th month. At this time-point the cumulative body weight gain in high-dose females was 23% lower as compared to controls

There were no treatment-related effects in females fed 2000 or 8000 ppm glyphosate.

Table B.6.5.8-2 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of cumulative body weight changes

	Males				Females			
ppm	**0**	**2000**	**8000**	**20000**	**0**	**2000**	**8000**	**20000**
Number of animals	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Body weight (g)								
Week 1	319.1	325.8	322.5	325.0	237.9	238.2	240.9	238.8
Week 7	478.0	489.1	470.4	466.1	296.8	298.9	299.4	**287.7***
Week 53	715.6	784.5	739.0	742.4	447.7	457.0	458.0	424.2
Week 104	672.8	747.3	711.9	728.6	488.2	535.6	542.6	471.4
Bodyweight gain								
Week 1	35.1	**41.9***	38.7	**41.0***	17.0	17.4	20.1	18.1
Week 1-7	194.0	205.2	186.5	182.1	78.9	70.2	78.6	**67.0** ** **(-15%)**
Week 53	468.4	500.7	455.8	458.4	226.4	236.3	237.1	203.0
Week 104	395.3	466.7	436.0	440.6	267.3	315.4	323.6	250.3

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

E. Food consumption and compound intake

There were no statistically significant decreases in food consumption in any group of either sex during the study period. However, significant increased food consumption was noted frequently in high-dose males, and on some occasions in low-dose males.

Table B.6.5.8-3 Chronic study of glyphosate administered in feed to Albino rats ([redacted] *et al.*, 1990): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	**Females**
1 (control)	0	0	0
2 (low)	2000	89	113
3 (mid)	8000	362	457
4 (high)	20000	940	1183

F. OPHTHALMOSCOPY

There were no treatment-related ocular effects observed in females of any dose group, as well as of males of the low-, and mid-dose group. In high-dose males a statistically increased incidence ($p \leq 0.05$) of cataractous lens changes were observed at the ophthalmic examination prior to termination although the observed incidence of 25% was within the historical control range of 0-33 %. A second independent ophthalmic examination also performed prior to termination confirmed a statistically significant increase ($p \leq 0.05$) in the incidence of cataractous lens changes in high-dose males (1/14 (control) compared to 8/19 (high dose)). The results are summarised in the table below.

Table B.6.5.8-4 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

	Dose group (ppm in diet)*			
	0	**2000**	**8000**	**20000**
1st examination	0/15 (0%)	↑1/22 (5%)	↑3/18 (17%)	↑**5/20**** **(25%)**
2nd examination	1/14 (7%)	↑2/22 (9%)	↑3/17 (18%)	↑**8/19**** **(42%)**
HCD (range) [%]	0 - 33			

* Number of rats affected/ number of rats examined (% affected);

** Statistically significant from control ($p \leq 0.05$)

The histopathological examination confirmed a slightly, but not statistically, increased incidence of degenerative lens changes (i.e. cataract and/or lens fibre degeneration) in high-dose males (see table below).

Table B.6.5.8-5 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Histopathological confirmed incidences of cataract and lens fibre degeneration in males

	Dose group (ppm in diet)*			
	0	**2000**	**8000**	**20000**
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

To summarise, ophthalmic examinations performed at the end of the study revealed a statistically significant increase in the incidence of degenerative lens changes in high dose males. Histopathological examination also indicated a slightly increased incidence of degenerative lens changes in high dose males, although the difference was not statistically significant. Interpretation of these data are difficult since the numbers of animals examined ophthalmologically and affected at the end of the study were small. Nonetheless, the occurrence of degenerative lens changes in high dose male rats appears to have been exacerbated by treatment. There is no indication of treatment-related ocular effects in low or mid dose males or in any group of treated females.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology and clinical chemistry evaluations noted various changes in the examined parameters. However, the changes were not consistently noted at more than one time point, were small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant.

The statistically increased alkaline phosphatase level observed in high-dose females at termination was mostly due to an extremely high value for one animal. However, this finding is in line with observation made in other long-term studies in rats and therefore considered to be treatment related.

Table B.6.5.8-6 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of haematological data

ppm		Males				Females			
		0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
RBC									
Day 191-192	Mean [10^6/mm²]	8.8054	↓8.7839	↓8.3054	↓8.0196	7.9250	↑8.1964	↑8.0159	↑8.3679
	Std. Dev.	0.9199	0.6651	1.2732	1.7048	0.4517	0.6335	0.5570	0.4893
	% control	-	100	94	91	-	103	101	106
Day 370-374	Mean [10^6/mm²]	8.3536	↑8.4321	↓8.1964	↓7.8250	7.4385	↑7.6875	↓6.4518	↑7.7339
	Std. Dev.	0.5558	0.3910	0.4997	0.5477	0.5010	0.3567	1.7354	0.4289
	% control	-	101	98	94	-	103	87	104
Day 569-570	Mean [10^6/mm²]	7.1671	↑**8.9484****	↑7.8607	↑8.1786	8.1429	↓7.3607	↓7.2911*	↓**7.4625***
	Std. Dev.	1.5571	**0.4913**	0.6785	0.6557	0.6838	1.6918	0.8798	**0.6544**
	% control	-	**128**	111	115	-	90	90	**92**
Day 734-	Mean [10^6/mm²]	6.8714	↓5.8946	↑7.9536	↓6.5571	6.3857	↓6.0839	↑6.4589	↓5.3911
	Std. Dev.	1.5015	1.4206	1.8201	1.7326	1.0665	1.1168	0.5965	1.4334

Table B.6.5.8-6 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of haematological data

	ppm	Males				Females			
		0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
735	% control	-	86	116	95	-	95	101	84
HGB									
Day 191-192	Mean [g/dL]	16.8571	↓16.8214	↓15.8214	↓15.5000	16.0714	↑16.8571	↑16.4881	↑**17.2857***
	Std. Dev.	1.0487	0.8367	2.4603	2.8865	0.7192	1.0950	1.1573	**0.7520**
	% control	-	98	97	92	-	105	103	**108**
Day 370-374	Mean [g/dL]	16.2500	↓15.9107	↓15.8214	↓**15.2143***	15.5754	↑16.8214	↓13.6786	↑16.1786
	Std. Dev.	0.7761	0.5549	0.5905	**1.1869**	1.0891	0.8996	2.8412	0.8213
	% control	-	98	97	**94**	-	102	88	104
Day 569-570	Mean [g/dL]	13.6508	↑**16.8452****	↑**15.7679***	↑**16.1260***	16.6429	↓15.5000	↓15.7321	↓16.2321
	Std. Dev.	2.3113	**0.6973**	**0.7809**	**1.0243**	0.8325	2.9207	1.4688	1.2007
	% control	-	**90**	**117**	**100**	-	100	105	92
Day 734-735	Mean [g/dL]	13.3036	↓12.0179	↑15.6071	↓13.2857	13.5357	↑13.6000	↑14.2143	↓12.4821
	Std. Dev.	2.4358	2.9439	2.5200	2.6409	1.6297	1.8485	1.3101	2.3198
	% control	-	90	117	100	-	100	105	92
HCT									
Day 191-192	Mean [%]	48.8393	↓48.4464	↓45.6250	↓45.4643	46.1964	↑47.9643	↑47.2421	↑**49.5357***
	Std. Dev.	3.7127	3.1509	6.1100	8.8209	2.7626	3.4485	.32453	**1.9673**
	% control	-	99	93	93	-	104	102	**107**
Day 370-374	Mean [%]	47.1071	↓47.0893	↓46.2500	↓44.2500	44.4841	45.3929	39.2143	46.2143
	Std. Dev.	2.7585	2.2225	2.4943	4.1902	3.2477	2.6684	8.4267	2.6147
	% control	-	100	98	94	-	102	88	104
Day 569-570	Mean [%]	40.4960	↓**49.3452****	↑44.4286	↑45.4464	47.7143	↓44.1964	↓44.1786	↓**43.8571***
	Std. Dev.	6.9710	**2.8202**	4.1869	3.1274	3.2240	8.1277	3.7136	**3.3674**
	% control	-	**122**	110	112	-	93	93	**92**
Day 734-735	Mean [%]	38.1429	↓34.1429	↑45.5000	↓37.7679	38.3750	↓36.8393	↑39.0893	↓34.4107
	Std. Dev.	7.3782	7.7766	9.4973	8.6534	4.8126	5.3253	2.8228	7.1770
	% control	-	91	119	99	-	96	102	90
MCH									

Table B.6.5.8-6 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of haematological data

ppm		Males				Females			
		0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Day 191-192	Mean [pg]	19.2300	19.2300	↓19.0700	↑19.5200	20.3100	↑20.6000	↑20.5444	↑20.6500
	Std. Dev.	1.0446	0.8945	0.9129	1.1104	0.5646	0.4967	0.4391	0.6621
	% control	-	100	99	102	-	98	103	100
Day 370-374	Mean [pg]	19.5300	↓18.9200	↓19.3400	↓19.4800	20.9667	↓20.5800	↑21.6600	↓20.9400
	Std. Dev.	0.9129	0.5534	0.8592	0.5007	0.5000	0.6106	1.9614	0.6484
	% control	-	97	99	100	-	98	103	100
Day 569-570	Mean [pg]	19.5111	↓18.8667	↑20.1600	↑19.7400	20.5000	↑21.3700	↑**21.6400***	↑**21.7700****
	Std. Dev.	1.6534	0.8170	1.5657	0.7276	1.1086	1.5326	**1.3142**	**0.9393**
	% control	-	97	103	101	-	104	**106**	**106**
Day 734-735	Mean [pg]	19.5600	↑20.4100	↑19.9400	↑20.5300	21.3900	↑22.4000	↑22.0500	↑23.7800
	Std. Dev.	1.3906	1.0386	1.9968	1.8655	1.6650	1.7951	0.7472	2.8790
	% control	-	104	102	105	-	105	103	111
MCHC									
Day 191-192	Mean [g/dL]	34.5900	↑34.8400	34.5900	↓34.1800	34.8600	↑35.1500	↓34.8333	34.8600
	Std. Dev.	0.8543	0.9095	1.5495	0.8080	1.0146	0.7457	0.4359	0.6186
	% control	-	101	100	99	-	101	100	100
Day 370-374	Mean [g/dL]	34.6000	↓33.9100	↓34.2400	↓34.5500	35.1333	↓34.8700	↓34.9500	↓35.0400
	Std. Dev.	0.8832	0.8425	1.1247	1.1128	1.2903	0.4668	1.1414	0.8579
	% control	-	98	99	100	-	99	99	100
Day 569-570	Mean [g/dL]	33.8333	↑34.1889	↑35.7000	↑35.4500	34.9200	↑35.0400	↑35.5200	↑**37.0400****
	Std. Dev.	1.1413	1.4243	2.6658	0.8410	1.1622	0.5358	1.2770	**1.7430**
	% control	-	101	106	105	-	100	102	**106**
Day 734-735	Mean [g/dL]	34.9900	↓34.5400	↑34.6100	↑35.4000	35.3400	↑36.6900	↑36.3600	↑36.5700
	Std. Dev.	2.5484	2.5439	2.3483	2.2420	2.3220	2.6710	1.8001	2.8418
	% control	-	99	99	101	-	104	103	103
PLT									
Day 19	Mean [1000/mm²]	794.1071	↑830.3571	↓316.9643	↑794.8214	821.0714	↓759.6429	↓818.4524	↓810.7143

Table B.6.5.8-6 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of haematological data

ppm		Males				Females			
		0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
1-192	Std. Dev.	129.4230	100.2371	104.5867	212.9330	113.8285	88.3811	127.0276	174.4830
	% control	-	105	103	100	-	93	100	99
Day 370-374	Mean [1000/mm²]	765.8928	↑835.5357	↑825.7143	↑767.1428	774.6031	↓760.7143	↑857.3214	↓763.9286
	Std. Dev.	182.1671	75.6840	119.3069	166.2584	73.7771	71.1004	161.4499	98.1865
	% control	-	109	108	100	-	98	111	99
Day 569-570	Mean [1000/mm²]	1180.3572	↓**847.4207****	↓**855.3571****	↓**857.1429****	733.5714	↓727.5000	↓733.2143	↑735.000
	Std. Dev.	320.4399	**160.6674**	**136.1548**	**148.8952**	171.3310	114.1405	79.9713	122.7554
	% control	-	**72**	**72**	**73**	-	99	100	100
Day 734-735	Mean [1000/mm²]	1300.1984	↓1142.0635	↓1025.3572	↓1166.4286	804.6428	↓781.6071	↑837.8571	↑835.6548
	Std. Dev.	354.3073	312.2577	295.7942	304.2532	188.888	123.2532	120977 8	174.9873
	% control	-	88	79	90	-	86	93	92

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Table B.6.5.8-7 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of serum chemistry data

ppm		Males				Females			
		0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Chloride									
6 month	group means	107	107	105	105	105	107	104	106
12 month		103	103	103	103	99.5	99.7	100	98.7
18 month		107	**104***	**105***	**102****	97.2	99.8	97.3	97.2
24 month		145.6	**145.2**	**141.3**	**195.8**	144.2	121.7	169.3	118.4
Tot Bili									
6 month	group means	0.11	0.11	0.11	0.10	0.15	0.17	0.15	0.18
12 month		0.09	0.10	0.12	0.12	0.17	**0.11***	0.13	0.14
18 month		0.13	0.19	0.29	0.16	0.16	0.13	0.17	0.13
24 month		0.07	0.10	0.12	0.10	0.10	0.09	0.11	0.09
Tot Protein									
6 month	group means	7.9	7.8	7.7	7.5	8.9	8.9	8.9	8.4
12 month		7.0	6.7	6.7	6.7	8.1	7.7	**7.4***	7.8
18 month		7.2	**7.9***	**7.9***	7.7	7.9	7.7	7.4	7.4
24 month		6.67	6.96	6.79	6.56	7.49	7.29	7.28	6.96
Albumin									
6 month	group means	4.0	3.8	3.8	3.8	4.9	4.8	4.9	4.5
12 month		3.4	3.2	3.3	3.3	4.4	4.1	**4.0***	4.2
18 month		2.9	**3.2***	3.1	3.2	4.0	3.9	3.8	3.9

Table B.6.5.8-7 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of serum chemistry data

		Males				Females			
ppm		**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
24 month		3.20	3.43	3.50	3.38	4.22	4.41	4.77	4.11
Phos									
6 month	group means	8.4	7.8	7.4	7.4	7.1	7.9	7.0	7.5
12 month		6.9	7.6	7.5	6.6	6.1	6.4	6.0	6.2
18 month		6.4	6.0	5.8	**5.6****	5.6	5.4	5.3	5.2
24 month		8.61	9.04	**7.49****	8.15	7.92	7.77	7.61	8.19
Alk Phos									
6 month	group means	209	201	262	248	80.5	83.7	78.6	89.7
12 month		169	177	199	209	55.2	62.5	71.6	67.0
18 month		205	225	210	254	56.4	94.9	101	75.8
24 month		180.8	147.5	177.9	188.3	95.5	70.8	87.8	**178.5* (+87%)**
Creat									
6 month	group means	0.52	0.48	0.52	0.45	0.72	0.68	0.68	0.71
12 month		0.47	0.47	0.47	0.50	0.54	0.50	0.43*	0.52
18 month		0.64	0.63	0.66	0.53	0.53	0.54	0.52	0.50
24 month		0.83	0.98	0.68	0.82	0.75	0.66	0.67	0.62
Chol									
6 month	group means	42.5	35.8	43.3	37.1	59.2	55.1	64.9	49.1
12 month		49.1	40.8	44.3	56.6	49.6	53.3	68.5	56.2
18 month		87.4	88.1	81.3	98.0	104	**69.7***	77.8	**60.9****
24 month		145.6	145.2	141.3	196.8	144.2	121.7	109.3	118.4
Potassium									
6 month	group means	6.7	6.8	6.6	6.7	6.4	6.4	6.0	6.6
12 month		6.6	7.6	7.7	6.9	6.3	5.9	6.0	6.4
18 month		6.0	6.0	6.2	6.4	4.6	5.2	**5.5****	5.1
24 month		6.72	6.62	6.12	5.77	6.18	6.59	6.25	6.43

*/** Significantly different from the control at 5 % and 1 % level of proability, respectively.

H. URINALYSIS

Urine specific gravity was statistically significant increased at the Month 6 examination in males. The observed statistically significant decreased urinary pH at 6, 18 and 24 months might be related to the renal excretion of glyphosate, which is an acid.

Table B.6.5.8-8 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of urinalysis data

		Males				Females			
ppm		**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Specific gravity									
6 month	Group mean	1.043	↑1.046	↑1.047	↑**1.061***	1.056	↓1.053	↓1.049	↑1.064
	Std. Dev.	0.011	0.008	0.014	0.022	0.014	0.022	0.012	0.027
12 month	Group mean	1.038	↓1.029	↓1.033	↑1.039	1.035	↑1.037	↑1.036	↑1.040
	Std. Dev.	0.011	0.008	0.004	0.010	0.007	0.012	0.006	0.012
18 month	Group mean	1.053	↑1.057	↓1.052	↑1.056	1.034	↑1.041	↓1.032	↑1.040
	Std. Dev.	0.018	0.011	0.012	0.013	0.006	0.011	0.009	0.026
24 month	Group mean	1.034	1.034	↑1.036	↑1.039	1.031	↑1.032	↑1.032	↑1.034
	Std. Dev.	0.013	0.009	0.008	0.012	0.005	0.009	0.007	0.012
pH									
6 month	Group mean	6.9	↓6.5	↓6.8	↓**6.0***	5.6	↑5.9	5.6	↓5.4
	Std. Dev.	0.4	0.4	0.6	1.1	0.4	0.6	0.3	0.4

Table B.6.5.8-8 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of urinalysis data

		Males				Females			
ppm		0	2000	6000	20000	0	2000	6000	20000
Specific gravity									
12 month	Group mean	6.6	↓6.5	6.6	↓6.2	5.8	↑6.0	5.8	↑6.0
	Std. Dev.	0.4	0.5	0.5	0.4	0.4	0.4	0.3	0.3
18 month	Group mean	6.8	↓6.2	↓6.4	↓**5.8****	6.2	↑6.4	6.2	↓5.8
	Std. Dev.	0.9	0.4	0.6	0.5	0.5	0.4	0.4	0.5
24 month	Group mean	6.4	6.0	6.0	↓**5.7***	5.8	↑6.2	↑6.0	5.8
	Std. Dev.	0.6	0.5	0.6	0.4	0.4	0.5	0.6	0.5

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

I. NECROPSY

Gross pathology

There were no treatment-related gross pathological findings observed at necropsy.

Organ weights

At interim kill after 12 months relative liver weights were slightly, but statistically significantly increased in high-dose males. At terminal sacrifice absolute liver weights, as well as liver to brain weight ratios were also statistically increased in high-dose males. Relative liver weights when compared to bodyweight were not significantly different compared to the control. There were no other significant and dose-related effects on organ weights.

Table B.6.5.8-9 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of organ weights

ppm		Males				Females			
		0	2000	6000	20000	0	2000	6000	20000
Terminal body weight									
Day 370 - 374	Mean	720.3800	↑722.0500	↑726.7900	↓691.4000	403.8200	↑455.0000	↑437.2250	↓386.3400
	Std. Dev.	78.0037	125.5382	85.4748	85.0447	84.0449	70.7029	89.8582	52.0690
	% control	-	100	101	96	-	113	108	95
Day 734 - 737	Mean	632.9286	↑**750.4526***	↑672.5059	↑705.6647	476.0409	↑522.2500	↑512.4824	↓437.9667
	Std. Dev.	93.8606	**114.6882**	120.9860	148.6255	116.0952	93.2104	117.9763	109.1727
	% control	-	**119**	106	111	-	110	108	92
Liver - absolute									
Day 370 - 374	Mean	17.4555	↑18.1414	↑18.3410	↑18.6718	11.6039	↑12.4752	↑13.8455	↓11.3471
	Std. Dev.	3.2018	3.4751	2.4805	2.2841	2.2199	1.9673	2.7471	2.3439
	% control	-	104	105	107	-	108	119	98
Day 734	Mean	16.5051	↑17.9773	↑17.6834	↑**18.6139***	14.9135	↑15.2995	↓14.3320	↓14.9291
	Std. Dev.	2.3151	1.9917	1.9964	**2.4702**	3.2161	3.0758	3.3668	4.5552

Table B.6.5.8-9 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Summary of organ weights

ppm		Males				Females			
		0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
- 737	% control	-	109	107	**113**	-	103	96	100
Liver weight – relative to brain weight									
Day 370 - 374	Mean	800.44	814.75	835.12	846.15	548.28	599.70	666.88	554.64
	Std. Dev.	132.57	127.24	127.04	93.33	109.89	95.25	128.44	115.03
	% control	-	102	104	106	-	109	122	101
Day 734 - 737	Mean	707.30	783.46	753.27	**805.70***	701.84	718.03	667.90	700.05
	Std. Dev.	105.65	99.72	87.25	**95.79**	161.68	138.16	157.86	198.51
	% control	-	111	106	**114**	-	102	95	100
Relative liver weight									
Day 370 - 374	Mean	2.4082	2.5155	2.5269	**2.7122***	2.8898	2.7545	3.2157	2.9321
	Std. Dev.	0.2293	0.2240	0.2026	**0.2791**	0.2380	0.2838	0.5918	0.3157
	% control	-	104	105	**1.13**	-	95	111	101
Day 734 - 737	Mean	2.6481	2.4586	2.5945	2.7384	3.2657	2.9898.	2.8607	3.4365
	Std. Dev.	0.4478	0.5238	0.5044	0.6448	0.8401	0.6508	0.5570	0.6152
	% control	-	93	98	103	-	92	88	105

*/** Significantly different from the control at 5 % and 1 % level of probability, respectively.

Histopathology

Non-neoplastic lesions

Apart from the eye findings mentioned above histopathological examination showed only one other lesion that reached statistical significance. This was an increased incidence of inflammation of the stomach squamous mucosa in females fed 8000 ppm glyphosate (see table below). Although not strictly dose-related it is considered that a treatment related effect cannot be excluded consider the increase in both hyperplasia and inflammation.

Table B.6.5.8-10 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Incidence of inflammation and hyperplasia of the stomach squamous mucosa

		Dose group (ppm in diet)*			
		0	2000	8000	20000
Males	Inflammation	2/58	3/58	5/59	7/59
	Hyperplasia	3/58	3/58	4/59	7/59
Females	Inflammation	0/59	3/60	9/60**	6/59
	Hyperplasia	2/59	3/60	7/60	6/59

* Number of rats affected / number of rats examined

** Statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality)

Neoplastic lesions

The only statistically significant difference in neoplastic lesions was an increased incidence of pancreatic islet cell adenomas observed in low-dose males (see **Table B.6.5.8-11**). The incidence (14%) in low-dose males was outside the historical control range (1.8 – 8.5%) for this laboratory, but was in the historical control range ≥17%) observed in reports from other laboratories. The RMS notes that the use of historical control data from other laboratories is generally not accepted. However, there was no dose-related trend for this finding in the male groups, as indicated by the lack of statistical significance in the Peto trend test. And due to the lack of a dose-related proliferative effect (hyperplasia) and or progression (carcinoma) of this lesion was seen, and as such effects were not observed in females, this finding was not considered to be treatment-related. Pancreatic islet cell adenomas are further discussed in Volume 1 (Section 2.6.5.1).

Table B.6.5.8-11 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990): Incidence of pancreatic islet cell findings

Finding	Sex	Dose group (ppm in diet)*			
		0	2000	8000	20000
Hyperplasia	Males	2/58	0/57	4/60	2/59
	Females	4/60	1/60	1/60	0/59
Adenoma	Males	1/58	8/57**	5/60	7/59
	Females	5/60	1/60	4/60	0/59
Carcinoma	Males	1/58	0/57	0/60	0/59
	Females	0/60	0/60	0/60	0/59

* Number of rats affected / number of rats examined

** Statistically significant at $p \leq 0.01$ (Fisher exact test with Bonferroni inequality)

Besides this effect on pancreatic islet cells, the RMS also noted an apparent increase in hepatocellular adenomas in high dose males which was not reported to be statistically significant. Furthermore, an apparent increase was noted in thyroid C-cell adenomas in mid and high dose animals of both sexes. The increase was not statistically significant according to the study report. The relevance of the carcinogenicity findings in the context of the classification of glyphosate is discussed in Volume 1

In addition, an potential increase in skin keratoacanthomas was noted in males in the publication by Portier, 2020 (reported in B.6.5.18.2) although there is no linear dose response relationship. Upon statistical re-analysis by an external statistician, a trend test was performed for the skin keratoacanthomas. There was no significant trend observed (P (two-sided) = 0.15 for the extended Mantel-Haenszel test (stratified Cochran-Armitage trend) test)). The relevance of the carcinogenicity findings in the context of the classification of glyphosate is discussed in Volume 1.

Table B.6.5.8-12 Chronic study of glyphosate administered in feed to Albino rats ([redacted] et al., 1990) : Incidence of hepatocellular and thyroid tumours

Finding	Sex	Dose group (ppm in diet)*			
		0	2000	8000	20000
Hepatocellular adenoma	Males	3/60	2/60	3/60	8/60
	Females	6/60	2/60	6/60	1/60
Hepatocellular carcinoma	Males	3/60	2/60	1/60	2/60
	Females	1/20	0/60	1/60	2/60
Thyroid C-cell adenoma	Males	2/60	4/58	8/58	7/60
	Females	2/60	2/60	6/60	6/60
Thyroid C-cell carcinoma	Males	0/60	2/58	0/58	1/60
	Females	0/60	0/60	1/60	0/60
Skin keratoacanthomas	Males	1/59	3/60	4/60	5/59

* Number of rats affected / number of rats examined

Assessment and conclusion by applicant: In conclusion, glyphosate was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 20000 ppm for 24 months (corresponding to 940 mg/kg bw/day in males and 1183 mg/kg bw/day in females). The NOAEL for toxicity is 8000 ppm (corresponding to 362 mg/kg bw/day in males and 457 mg/kg bw/day in females), based on reduced body weights in females and cataract lens changes in males at 20000 ppm.

Assessment and conclusion by RMS:
Based on the stomach mucosal irritation observed at 8000 and 20000 ppm the NOAEL is concluded to be 2000 ppm (equal to 89 mg/kg bw/day in males and 113 mg/kg bw/day in females).

B.6.5.9. Long-term toxicity – rat, study 9

Data point:	CA 5.5/011
Report author	[redacted]
Report year	1981
Report title	A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats
Report No	77-2062
Document No	M-645914-01-1
Guidelines followed in study	None; in general accordance with OECD 451
Deviations from current test guideline (OECD 451, 2018)	The following deviations were noted from the current OECD guideline : - Histopathological examinations of the cervix, coagulating glands, gall bladder, Harderian gland, lacrimal glands, rectum, and vagina were not performed; - Dose levels selected were too low according to the recommendations given in OECD TG 451 and are low compared to the other studies.
Previous evaluation	Not accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant : Invalid, Category 3b Conclusion AGG : Since the dose levels in the study were too low the study is considered to be unacceptable. It is also noted that the quality of the study report is low and at times unreadable.

This study, was designed to assess the toxicity of glyphosate (ROUNDUP® Technical) when administered orally *via* the diet to 300 Sprague-Dawley (CD) rats (50/sex/group).

During the first week of the study, the test substance was administered at dose levels of 30, 100 and 300 ppm. For the remainder of the study, dose levels of 3.05, 10.30 and 31.49 mg/kg bw/day for the males, and 3.37, 11.22 and 34.02 mg/kg bw/day for the females were maintained.

Clinical laboratory studies were performed at months 4, 8, 12, 18 and 24. Water consumption was measured over two 3-day periods at months 18 and 24. All male and female groups were terminated at 26 months, at which time survival had decreased to 30 % in one group/sex. Select organs were weighed and organ/body and organ/brain weight ratios were calculated. Histopathological evaluations were performed on all animals dying spontaneously, sacrificed in a moribund condition and sacrificed at terminal necropsy.

During most of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. However, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination, it is not considered to be toxicologically significant.

The treated females showed no statistically significant differences in mean body weights as compared to the controls through Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in group mean body weights, especially groups II and III, although not in a dose-related fashion. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination of the study.

Evaluations of mortality, food consumption and water consumption data, haematology, clinical chemistry, urinalysis and terminal organ and body weights, organ/body weight ratios and organ/brain weight ratios failed to reveal any effect attributable to the administration of glyphosate.

The incidence of interstitial cell tumours of the testes in the group IV males was elevated compared to the controls. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the incidence in the group IV males is within the range observed in recent historical control data. In addition, the data suggest that the incidence in groups II through IV is within the normal biological variation observed for tumours at this site in this strain of rat. Where gross and microscopic changes occurred sporadically in the control and/or treated rats and were considered unrelated to the administration of the test substance.

I. MATERIAL AND MEDTHODS

A: Materials

1. Test material:

Identification:	Glyphosate (ROUNDUP® Technical)
Description:	Fine, white powder
Lot/Batch #:	XHJ-64
Purity:	98.7 %
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (CD®)
Source:	[redacted]
Age:	Approx. 6 weeks (at start of study)
Sex:	Males and females
Weight at dosing:	Males: approx. 124.0 g; females: approx. 102.3 g
Acclimation period:	12 days
Diet/Food:	Standard laboratory diet (Purina Lab Chow®), *ad libitum*
Water:	Automated water system (Elizabethtown Water Company), *ad libitum*
Housing:	Individually in elevated stainless steel cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1978-07-12 to 1980-08-26 (males); 1978-07-12 to 1980-09-04 (females)

Animal assignment and treatment:

In a lifetime feeding study groups of 50 Sprague-Dawley rats per sex received daily dietary doses of 0, 30, 100 and 300 ppm glyphosate (equivalent to mean achieved dose levels of 0, 3.05, 10.30 and 31.49 mg/kg bw/day for males and 0, 3.37, 11.22 and 34.02 mg/kg bw/day for females) for 26 months.

Clinical observations
All rats were examined for mortality and gross signs of toxicological or pharmacological effects twice daily. Detailed physical examinations for signs of local or systemic toxicity, pharmacologic effects and palpation for tissue masses were conducted weekly.

Body weight
Individual body weights were recorded twice prior to start of treatment, at weekly intervals from Week 1 to 14, biweekly thereafter and terminally (after fasting).

Food consumption and compound intake
Food consumption was recorded once prior to start of treatment, at weekly intervals for the first 14 weeks, and biweekly thereafter. The test substance intake was calculated from food consumption data. Based on nominal concentrations.

Water consumption
Water consumption was measured at month 18 and 24 over 2-three day periods.

H**aematology and clinical chemistry**
Blood was obtained *via* venepuncture of the orbital sinus (retrobulbar venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Rats were fasted overnight prior to blood collections and were not dosed until after samples were collected. Blood was collected from 10 animals per sex and group at Months 4, 8, 12, 18 and 24. The following parameters were measured: haematocrit, haemoglobin, total erythrocyte count, platelet count, total leukocyte count, differential leukocyte count. The parameters for clinical chemistry were: serum glutamic oxaloacetic transaminase (AST), serum glutamic pyruvic transaminase (ALT), alkaline phosphatase (ALP) blood urea nitrogen, glucose, lactic acid dehydrogenase, total cholesterol, total bilirubin, direct bilirubin, total protein, albumin, globulin, inorganic phosphorus, calcium, potassium.

Urinalysis
Individual urine samples were collected from the same animals as those used for haematology analyses at Month 4, 12, 18 and 24. The following parameters were determined: appearance, specific gravity, pH, glucose, ketones, protein, bilirubin, occult blood and microscopic analysis.

Sacrifice and pathology
A gross necropsy was conducted on all surviving animals at scheduled sacrifice after 26 month and on all animals that died spontaneously or were killed in a moribund condition. The following organ weights were determined: Adrenals, brain, gonads, heart, kidneys, liver, pituitary, spleen and thyroid.

Tissue samples were taken from the following organs and subjected to a histopathological examination: adrenals, aorta, blood smear, bone & bone marrow, brain, caecum, colon, duodenum, eyes, heart, head, ileum, jejunum, kidneys, liver, lung, lymph nodes (mesenteric and mediastinal), mammary gland (right inguinal), oesophagus, ovaries, pancreas, pituitary, prostrate, sciatic nerve, seminal vesicles, skeletal muscle, skin (with mammary gland), spinal cord (cervical, lumbar), spleen, stomach, mandibular salivary gland, testes with epididymides, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus.

Statistics
Body weight, food consumption, haematology and clinical chemistry parameters, organ weights and organ/body weight ratios and organ/brain weight ration were analysed. Mean values of all dose groups were compared to control at each time interval.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine

which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case (i.e. equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

The stability analyses were in the responsibility of the sponsor.

B. MORTALITY

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. Thereafter, significant reductions in the number of surviving animals occurred in all groups in roughly an equivalent fashion, culminating in the termination of the study at Month 26. At this time, survival had decreased to 30 % in the group I males and the group IV females, requiring that the study be terminated to ensure a sufficient number of animals at the terminal necropsy. At 24 months, survival levels equalled or exceeded 50 %, which is comparable to historical control data for rats of this strain.

Table B.6.5.9-1 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Percentage survival at termination after 26-month dietary exposure to glyphosate

Sex	Dose group (ppm)			
	0	30	100	300
Male	35/50	24/50	34/50	24/50
Female	32/50	27/50	20/50	35/50

Number of dead animals/total number of animals

C. CLINICAL OBSERVATIONS

Physical observations noted during the course of this study included alopecia, excessive lacrimation, nasal discharge and rales. These findings were present in animals in all groups, both male and female, in approximately the same incidence and are common observations in the laboratory rat. Time of appearance and duration was approximately the same in all groups, including the controls. Therefore, it is concluded that the administration of the test substance did not significantly affect the physical condition of the animals on test in this study.

D. BODY WEIGHT

No statistically significant differences were noted among the mean body weights of the treated males as compared to the group I controls during the course of this study. However, during a portion of the growth period, a slight but consistent trend toward reduced body weights in the treated males was evident. The maximum decrease was approximately 6 % in the high dose males, occurring during Month 16. Thereafter, this difference decreased resulting in little difference in mean body weights between groups at termination. Because this effect was slight and not evident at termination of the study and did not affect survival, it is not considered to be toxicologically relevant.

No statistically significant differences in mean body weights were found among the treated females as compared to the controls through Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in mean body weights as compared to the control, although not in a dose-related fashion. The magnitude of the reduction ranged between 10-15 % with the greatest difference evident in groups II and III. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination of the study. This pattern is similar to that evident in the males.

The pattern of increasing body weights for the first 16 months, followed by a plateau and then a slight decline, observed in the males and females in group I is typical for the laboratory rat. In the treated animals, particularly in the group IV males and groups II through IV females, a slight delay in reaching the plateau phase was observed. This effect was not dose-related in the treated females and may be due to biological variation.

Table B.6.5.9-2 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Body weight data

ppm		Males				Females			
		0	**30**	**100**	**300**	**0**	**30**	**100**	**300**
Number of animals		**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Week 62	Mean body weight (g)	694.7 (N=47)	671.3 (N=48)	670.9 (N=46)	659.1	390.0 (N=49	-369.1 (N=49)	-368.4 (N=48	378.5 (N=48)
	Std. Dev.	83.7	85.4	66.7	66.3	71.3	-58.7	-53.9	63.6
	Std. Err.	12.2	12.3	9.8	9.4	10.2	-8.4	-7.8	9.2
Week 64	Mean body weight (g)	701.5	684.4	673	664.2	397.9	373.8	375.5	384.1
	Std. Dev.	84.0	80.4	74.1	66.6	73.9	61.7	56.6	61.4
	Std. Err.	Not readable in original report				Not readable in original report			
Week 66	Mean body weight (g)	709.7 (N=47)	690.6 (N=47)	684.6 (N=45)	671.5	404.4 (N=49)	377.1 (N=49)	381.1 (N=47)	391.3 (N=48)
	Std. Dev.	85.4	80.0	71.9	69.0	75.9	64.8	58.8	63.9
	Std. Err.	12.5	11.7	10.7	9.9	10.8	9.3	8.6	9.2
Week 74	Mean body weight (g)	730.3 (N=44)	708 (N=47)	696.3 (N=45)	NA[1]	426.6 (N=48)	398.2 (N=46)	398.1 (N=47)	410.7 (N=47)
	Std. Dev.	92.9	85.2	82.4		88.0	65.3	61.0	75.8
	Std. Err.	14.0	12.4	12.3		12.7	9.6	8.9	11.1
Week 76	Mean body weight (g)	733.4 (N=42)	718.1 (N=47)	708.6 (N=44)	696.7 (N=49)	436.7 (N=48)	410.7 (N=46)	404.1 (N=47)	409.8 (N=45)
	Std. Dev.	99.4	89.3	83.2	75.9	93.7	67.8	63.8	82.4
	Std. Err.	15.3	13.0	12.5	10.8	13.5	10.0	9.3	12.3
Week 78*	Mean body weight (g)	724.8 (N=32)	724.8 (N=35)	698.5 (N=34)	691.3 (N=39)	427.3 (N=47)	404.4 (N=36)	405.5 (N=37)	419.9 (N=33)
	Std. Dev.	104.2	95.6	84.7	78.9	86.9	71.4	65.3	87.3
	Std. Err.	18.4	16.2	14.5	12.6	14.3	11.9	10.7	15.2

The number in brackets represents the animal number of the respective dose/timepoint.

*At week 78 it seems that fewer number of animals were weighted compared to the previous and following weeks.

[1] Due to the poor quality of the study report the exact body weight could not be read.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption values relative to body weight were highest during the early stage of the study, gradually declining through Month 18. Thereafter these values remain essentially unchanged for the duration of the study. This pattern was observed in all groups of both sexes and is typical for long-term rat studies. Occasional statistically significant differences were noted in the treated animals of both sexes relative to their respective controls, but these differences in mean food consumption were slight and occurred sporadically unrelated to dose level. Therefore, it is concluded that the dietary administration of Glyphosate at the doses utilised in this study did not significantly affect food consumption values in either sex.

Test substance intake

During the first week of the study, the test substance was administered at the following dose levels for both sexes: 0, 30, 100 and 300 ppm for groups I through IV, respectively. Based on group mean food consumption, body weight values and nominal dietary concentrations for Week 1, test substance intake was calculated on a mg/kg/day basis. Thereafter, weekly through 14 weeks and biweekly for the duration of the study, the dietary levels of the test substance were adjusted to maintain the test substance intake attained for Week 1. These values were as follows: 0, 3.05, 10.30 and 31.49 mg/kg bw/day for groups I through IV males, respectively and 0, 3.37, 11.22 and 34.02 mg/kg bw/day for groups I through IV females, respectively. These values were maintained within a narrow range throughout the study.

Water consumption
Mean values for both the treated male and female groups were not significantly different from their respective control groups at the intervals studied. Thee data indicate that the administration of the test substance did not significantly affect water consumption in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology
Analysis of the group mean haematology data for both sexes indicates no toxicologically significant differences in any of the parameters evaluated. The few statistically significant differences noted appear to be due to random variation as no consistent treatment-related pattern is evident. For example, red blood cell count at 4 months was statistically reduced but in the low dose males only. In addition, an 24 months a decrease in WBC was noted but in mid dose females only. On the basis of this data it is concluded that the administration of the test substance did not affect the haematology parameters evaluated.

Clinical Chemistry
Mean clinical chemistry data for all groups of both males and females fall within the normal physiological range for the laboratory rat. Occasional statistically significant differences were noted, but these appear due to random fluctuation, as no treatment-related pattern emerged. For example, BUN was increased at 8 months in low and mid dose males but no effect was observed in high dose males. Thus, on the basis of this data, the administration of the test substance did not significantly affect any of the clinical biochemistry parameters evaluated during the course of this study.

H. URINALYSIS
No significant differences were noted in the urinalysis data when the control groups were compared to the treated groups for both sexes. Occasional values outside the normal range were found; however, these values occurred sporadically exhibiting no consistent pattern.

I. NECROPSY

Gross pathology
Gross observations noted at necropsy were similar in incidence between control and treated animals of both sexes. Lesions noted were those commonly found in chronic studies conducted in this laboratory on the same strain of rats.

Organ weights
No statistically significant differences were noted in the terminal organ weights, organ/body weight ratios and organ/brain weight ratios of the treated groups as compared to their respective controls. Slight differences in these parameters were observed in a group by group comparison; however, no consistent pattern related to the administration of the test substance was evident.

Histopathology

Non-neoplastic lesions
In general, a good correlation was found between gross lesions noted at necropsy and microscopic findings of those tissue sections. The incidence and severity of the microscopic findings were similar in the control and treated animals of both sexes. Among the more common findings were changes found in the kidneys and lungs, lesions frequently found in chronic rat studies. Therefore, these findings are considered unrelated to the administration of the test substance.

Neoplastic lesions
A variety of neoplasms were found in both the control and treated animals of both sexes. The most common tumours were found in the pituitary in both sexes. In the females, mammary gland tumours were the next most common neoplasm found. In general, the incidence of all neoplasms observed occurred in the treated and control animals to a similar degree, or occurred infrequently such that a treatment-related association could not be made. The only exception to the above was the incidence of interstitial cell tumours of the testes in male rats. The incidence of this neoplasm in both the scheduled terminal sacrifice animals as well as the total number of animals on test is presented along with available historical control data for comparison in the table below:

Table B.6.5.9-3 A Lifetime Feeding Study of Glyphosate (ROUNDUP® Technical) in Rats ([redacted] 1981): Neoplastic findings compared to historical control data

	Interstitial cell tumour of the testes			
[ppm]	**0**	**30**	**100**	**300**
Terminal sacrifice	0/15 (0%)	2/26 (8%)	1/16 (6%)	4/26 (15%)
All animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)
Historical control data: - One study (performed between 1980 and 1982) in the same strain and at the same testing facility - The incidence of testes interstitial cell tumours was 4/80 (5%) for all animals				

(): incidence [%]

Based on the results in the present study, the data suggest a treatment-related response with regard to the incidence of testicular interstitial cell tumours in male rats. This tumour, as is frequently the case, increases in frequency among older animals as can be seen from comparing the incidence in the animals surviving until termination to the total incidence. As there was a lack of details on the historical control data (HCD) provided in the study report, the applicant was requested to provide further details on the HCD. The applicant replied that only for one contemporary chronic/carcinogenicity rat study conducted with Sprague-Dawley rats appropriate historical control data could be retrieved as for the remaining studies the data has been discarded. In this concurrent study, which was performed between 1980 and 1982, the incidence of testes interstitial cell tumours was 4/80 (5%) among controls. However, as HCD of only one study is available, this is of very limited value. The only comparison that can be made based on this very limited HCD is that the incidence of this tumour in the control group males that is lower (0%) than observed in this concurrent historical control data set and that the incidence in top dose males that is higher. In addition, no dose-response effect is observed when all dose levels are taken into account. The relevance for this finding in the context of classification and labelling of glyphosate is further discussed in Volume 1.

Although not reported as statistically significant in the study report an apparent increase in pancreatic islet cell adenomas was observed in low dose males (0/50, 5/49, 2/50, 2/50 at 0, 30, 100 and 300 ppm). However, no dose-response relationship was observed. The relevance for this finding in the context of classification and labelling of glyphosate is further discussed in Volume 1.

Assessment and conclusion by applicant: In conclusion, glyphosate (ROUNDUP® Technical) was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 300 ppm for 26 months (corresponding to 31.49 mg/kg bw/day in males and 34.02 mg/kg bw/day in females). The NOAEL for toxicity is 300 ppm (corresponding to 31.49 mg/kg bw/day in males and 34.02 mg/kg bw/day in females), based on the absence of findings in body weight changes, haematology, clinical chemistry, organ weight data or histopathological examinations.

Based on the reporting deficiencies and the use of too low dose groups the outcome of the study is not considered acceptable for the hazard and risk assessment of glyphosate.

Assessment and conclusion by RMS:
No adverse effects were noted up to the highest dose tested. However, the study was concluded to be unacceptable due the low dose groups and the poor quality of the study report.

B.6.5.10. Long-term toxicity – mouse, study 1

Data point:	CA 5.5/012 CA 5.5/013 CA 5.5/014 CA 5.5/015

Report author	[redacted] *et al.*
Report year	2009
Report title	Glyphosate Technical: Dietary Carcinogenicity Study in the Mouse
Report No	2060-0011
Document No	NA
Guidelines followed in study	OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA OPPTS 870.4200 (1996)
Deviations from current test guideline (OECD 451, 2018)	The following deviations were noted compared to the current OECD guideline: - histopathological examinations of the cervix and the coagulating glands were not performed.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant : Valid, Category 2a Conclusion AGG : Some minor deviations were noted compared to OECD 451 which are not considered to affect the validity of the study. Therefore, the study was concluded to be acceptable.

The carcinogenic potential of glyphosate technical was assessed in an 18-month feeding study in male and female CD-1 mice. Groups of 51 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm Glyphosate technical (equal to 0, 71.4, 234.2 and 810 mg/kg bw/day in males and 0, 97.9, 299.5 and 1081.2 mg/kg bw/day in females). Observations covered clinical signs, body weight, food and water consumption, palpation of masses, organ weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues for all control and high dosage group animals killed at termination. In addition, differential white blood cell counts were performed for animals that were killed or died in extremis and for selected animals at twelve and eighteen month of treatment. The dose-levels were chosen based on available toxicity data.

There were no treatment-related deaths or clinical signs in any of the dose-groups. In the carcinogenicity study, survival after 78 weeks of treatment was 76, 80, 76 and 69 % in males and 73, 75, 75 and 78 % in females in the control through high dosage groups, respectively.

There were no treatment-related effects on body weight gain or food and water consumption noted. No significant treatment-related effects were noted on differential white blood cell counts in both sexes. There were no treatment-related trends in the proportion of masses observed, number of mice affected or time to appearance of palpable masses. Gross pathology, organ weight data revealed no treatment-related effects. Histopathological evaluation revealed an apparent increase in malignant lymphoma in males (0/51, 1/51, 2/51, 5/51 at 0, 500, 1500 and 5000 ppm). The relevance of this finding in the context of the classification of glyphosate is discussed in Volume 1.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	Glyphosate
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7%
Stability of test compound:	Expiry: 2008-03-25

2. Vehicle and/ or positive control:	Diet	
3. Test animals:		
Species:	Mouse	
Strain:	CD-1, Crl:CD-1 (ICR) BR	
Source:	[redacted]	
Age:	Approx. 5 – 6 weeks	
Sex:	Males and females	
Weight at dosing:	Males: 22 – 32 g, females: 18 – 28 g	
Acclimation period:	At least ten days	
Diet/Food:	Rat and Mouse SQC Ground diet No. 1, Special Diet Services Limited, UK), *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Initially in groups of three per sex in polypropylene solid-floor cages.	
Environmental conditions:	Temperature:	21 ± 2 °C
	Humidity:	55 ± 15 %
	Air changes:	at least 15/hour
	12 hours light/dark cycle	

B: Study design and methods

In life dates: 2005-10-10 to 2007-11-19

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 51 CD-1 mice per sex received daily dietary doses of 0, 500, 1500 and 5000 ppm (equal to 0, 71.4, 234.2 and 810 mg/kg bw/day in males and 0, 97.9, 299.5 and 1081.2 mg/kg bw/day in females) Glyphosate technical in diet. Additional 12 mice per sex, designated for veterinary controls, were housed and maintained alongside treated animals.

Test diets were prepared prior to start of treatment and then weekly by mixing a known amount of the test substance with a small amount of basal diet and blending for 19 minutes. This pre-mix was then added to larger amount of basal diet and blended for further 30 minutes.

The stability and homogeneity of the test material in diet were determined. Samples of each dietary admixture were analysed for achieved concentration monthly for the first six months and then every three months thereafter.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all mice and recorded weekly. Observations for morbidity, and mortality were made twice daily. Additional unscheduled examinations were performed on animals that showed ill-health.
All surviving animals were palped weekly for size, position and appearance of new or existing masses.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Body weights were also determined before sacrifice. Bodyweight data were reported only until Week 77.

Food consumption and compound intake
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. Food consumption data were reported only until Week 77. Food efficiency and compound intake was calculated from the recorded food consumption data.

Water consumption

Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes.

Haematology
Blood smear samples were collected 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.

Sacrifice and pathology
All animals that died or were killed in extremis during the conduct of the study, and all animals sacrificed t scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen and testes.

Tissue samples were taken from the following organs and preserved in buffered formalin: adrenals, aortic (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), gross lesions incl. palpable masses, head (incl. pharynx, nasopharynx and paranasal sinuses), heart, Harderian and lacrimal glands, ileum, jejunum, kidneys, larynx, liver and gall bladder, lungs (with bronchi), mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, preputial gland, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

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, tissues of the liver, lungs and kidneys, as well as gross macroscopic lesions and palpable masses from low and intermediate dose groups at termination were examined microscopically.

Statistics
All data were summarised in tabular form and analysed by computerised analysis using ProvantisTM Tables and Statistics Module. For each variable the of variance incorporating Student´s t-test and F-test. For each variable the most suitable transformation of data was found, the use of possible covariates checked and the homogeneity of means assessed using ANOVA or ANOVA and Bartlett´s test. The lowest treatment-related significant effects were determined using the Williams Test for parametric data or the Shirley Test for non-parametric data. If no response is found, but the data showed non-homogeneity of means, data were further analysed by a stepwise Dunnet (parametric) or Steel (non-parametric) test to determine significant differences from control. If required, pair-wise tests are performed using Students t-test (parametric) or the Mann-Whitney U test (non-parametric).

The levels of probability chosen as significant were $p < 0.01$** and $p < 0.05$*.

Histopathology data were analysed using Chi squared analysis (differences in the incidence of lesions occurring with an overall frequency of 1 or greater) and the Kruskal-Wallis one-way non-parametric analysis of variance (comparison of severity grades).

The levels of probability chosen as significant were $p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.1$.

Results

A. ANALYSIS OF DOSE FORMULATIONS
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 1 sample (500 ppm –level), which was +10 % of the nominal concentration.

The group mean achieved doses are summarised below.

Table B.6.5.10-1: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] *et al.*, 2009b): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Achieved dose level (mg/kg bw/day)*			
		Males		Females	
		Mean	Range	Mean	Range
1 (control)	0				
2 (low)	500	71.4	33 – 104	97.9	55 – 155
3 (mid)	1500	234.2	101 – 365	299.5	176 – 466
4 (high)	5000	810	461 - 1143	1081.2	610 - 1728

* based on actual food intake and body weight data

The results show a higher test material intake for females when compared to males for each dose level. Highest intakes were achieved within the first few treatment weeks, with subsequent decline thereafter.

B. MORTALITY

No treatment-related effects on the deaths occurred during the study, as well as no treatment-related effects on the time of death. From three male mice that were killed in extremis, examination results suggest that the morbidity of these animals was due to fighting between cage mates.

Table B.6.5.10-2: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse [redacted] *et al.*, 2009b): Cumulated mortalities after 78-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	500	1500	5000
Male	12 (6)	10 (8)	12 (6)	16 (6)
Female	14 (10)	13 (7)	13 (10)	11 (8)

() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5.10-3: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] *et al.*, 2009b): Percentage survival at termination after 78-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	500	1500	5000
Male	76	80	76	69
Female	73	75	75	78

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

There were no trends in the proportion of palpable masses observed during the study period. Based on the results (see **Table B.6.5.10-4**) no treatment-related effect on the development of palpable masses is seen for either sex. The slight increase in the mean number of masses per animal for high-dose females and mid-dose males was considered a coincidence. The median time to appearance of palpable masses was comparable for all dose groups of either sex.

Table B.6.5.10-4: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] *et al.*, 2009b): Group summary of palpable masses

Dose	Total number of animals in group		Number of animals with palpable masses		Total number of masses per group		Mean number of masses per animal		Median time (weeks) to appearance of masses	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀

Table B.6.5.10-4: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] *et al.*, 2009b): Group summary of palpable masses

Dose	Total number of animals in group		Number of animals with palpable masses		Total number of masses per group		Mean number of masses per animal		Median time (weeks) to appearance of masses	
0	51	51	28	23	45	38	0.88	0.75	42.00	45.75
500	51	51	↑32	↑28	↑49	↑49	↑0.96	↑0.96	42.00	↑46.08
1500	51	51	↑39	23	↑60	38	↑1.20	0.75	↑42.43	↓44.83
5000	51	51	↓25	23	↑49	↑51	↑0.96	↑1.00	↓41.67	↓42.50

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

Table B.6.5.10-5: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] *et al.*, 2009b): Body weight at termination after 78-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	**500**	**1500**	**5000**
Male	56.6	56.9	56.1	54.8
Female	44.5	42.4	42.7	42.2

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

F. WATER CONSUMPTION

There were no treatment-related effects on water consumption for either sex noted during the study.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

There were no significant treatment related effects on haematology parameters (white blood cell counts) that were consistently observed for animals of both sexes that persisted through the course of the study.

H. NECROPSY

Gross pathology

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.

Organ weights

There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

An apparent increase in malignant lymphoma was observed in males.

Table B.6.5.10-2: Glyphosate technical: Dietary Carcinogenicity Study in the Mouse ([redacted] *et al.*, 2009b): malignant lymphoma after 78-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	**500**	**1500**	**5000**
Male	0/51	1/51	2/51	5/51
Female	11/51	8/51	10/51	11/51

() number of animals killed in extremis

No historical control data (HCD) are available. HCD were requested for the purpose of this renewal but applicant informed that the data have been discarded.

The previous RAR of glyphosate (2015) also reported the following information:
There are more sources to support, based on historical control data, remarkable differences in the occurrence of malignant lymphoma in CD-1 mice. According to information obtained from the "Registry of Industrial Toxicology Animal-data" (RITA) database (Fraunhofer ITEM Institute, Hannover, Germany; https://reni.item.fraunhofer.de/reni/public/rita/,) and made available to the RMS only very recently by the GTF, male CD-1 mice had a mean incidence of 3.4% (of 470 animals in total) in the control groups from nine 18-/19-month long-term studies performed between 1994 and 1998. In the individual studies, incidences ranged from 0 up to 12%. In female mice, the mean control incidence was much higher (16.9% in a total of 350 examined animals). In line with that, actual study incidences in female mice varied between 4 and 32% (Anonym, 2015, ASB2015-2532).
For the Crl:CD1 (ICR) mouse [i.e., the strain that was used by [redacted] *et al. (2009, ASB2012-11492), in their glyphosate study],* [redacted] *(2010, ASB2015-2529 (note RMS: doc KCA 5.5-025 in current dossier)) reported data from a total of 13 (males) or 14 studies (females) with a duration between 78 and 104 weeks that had been performed between 2002 and 2006 by* [redacted]*. (Also this data was submitted by GTF following PRAS 125 meeting.) In males, malignant lymphoma was more rarely seen than in females since tumours of this type were found in the control groups in 8 out of 13 studies only with a minimum study incidence of 1/75 and a maximum one of 5/49 closely resembling that one at the top dose level of the* [redacted] *et al. (2009, ASB2012-11492) study with glyphosate. In female CD-1 mice, malignant lymphoma was observed in all but one of the 14 studies, even though with an extremely variable study incidence ranging from 2/60 up to 22/50.*
Based on their retrospective analysis of 20 long-term studies for carcinogenicity ([redacted] *1990-2002)* [redacted] *(2004, ASB2015-2533) described lymphoma as the most common tumour in young control CD-1 mice. This result was based on an analysis of premature deaths in these studies. In a total of 101 fatalities occurring up to week 50 of treatment in all these studies among male animals, lymphoma was found in 23 cases. In the 190 males which died between weeks 50 and 80 before scheduled termination, 36 were diagnosed with lymphoma. Among females, there were 68 premature deaths up to week 50 of which 19 had lymphoma suggesting a slightly higher rate than in males (28% vs. 23%). Between weeks 50 and 80, there were 211 deaths and, among them, 61 with lymphoma (ca 29% vs. 19% in males). It was noted that lymphoma incidence in the* [redacted] *colony was similar in females as in the ICR mouse (*[redacted]*, 2010, ASB2015-2529) or in CD-1 mice included in the RITA database (Anonym, 2015, ASB2015-2532) whereas a more frequent occurrence of this tumour type was noted in males. However, this might be due to a different focus of the analysis. In the RITA database and in the review from* [redacted]*, all animals on study were considered. In contrast,* [redacted] *(2004, ASB2015-2533) looked only at the premature deaths to which malignant lymphoma might have contributed to a rather large extent.*

The relevance of the apparent increase in malignant lymphoma in the context of the classification and labelling is discussed in Volume 1.

Assessment and conclusion by applicant: In conclusion, Glyphosate technical was not carcinogenic in the CD-1 mouse following continuous dietary exposure of up to 945.6 mg/kg bw/day (average for both sexes) for 18 months. The NO(A)EL for toxicity was 810 mg/kg bw/day for male mice and 1081 mg/kg bw/day for female mice, the highest dosage tested.
No non-neoplastic or neoplastic treatment-related findings were observed.
No increases in malignant lymphoma or salivary gland histopathological findings were observed.

Assessment and conclusion by RMS:
No adverse findings were observed up to the highest dose tested leading to a NOAEL of 810 mg/kg bw/day in males and 1081 mg/kg bw/day in females. It should be noted that the study was conducted as a carcinogenicity study and only a limited of other toxicity endpoints were included.

B.6.5.11. Long-term toxicity – mouse, study 2

Data point:	CA 5.5/016
Report author	[redacted]
Report year	2001

Report title	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
Report No	Toxi: 1559.CARCI-M
Document No	NA
Guidelines followed in study	OECD 451 (1981)
Deviations from current test guideline (OECD 451, 2018)	The following deviations were noted from the current OECD test guideline: - animals were observed for mortality only once per day; - food consumption was not measured monthly after the first 13 weeks; - histopathological examination of the cervix, Harderian gland, lacrimal gland, male mammary glands and vagina were not performed. Note from the applicant: The statistical evaluation of incidences of non-neoplastic and neoplastic lesions were evaluated using the Z-test, which is inappropriate for the analysis of tumour incidence data (the Z-test assumes a normal distribution). Peto's incidental tumour analysis was performed without assigning a Petocode to the neoplasms, which is inappropriate for the application of this test. The Cochran-Armitage test was only applied to the tumour incidences in the high dose group and the control group, which is inappropriate for a trend test, where all dose levels should be considered. To address these issues, it was decided to re-evaluate the statistical significance of all tumour incidence data, starting from the raw data tables of this report and applying appropriate statistical methods (see CA 5.5-017).
Previous evaluation	Yes, accepted in the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant : Valid Category 2a Conclusion AGG: It was noted while evaluating the histopathological findings in the individual animal data that quite a high incidence of ectoparasites on the skin and endoparasites in the GI-tract are reported which does put some doubts on the adequacy of the conducting lab. However, the occurrence of parasites is not expected to impact the tumour incidences in the study. The applicant pointed out that there were limitations to the study with regard to the statistical analysis used. To address this point the applicant submitted a new statistical analysis (see B.6.5.12.1 (CA 5.5-017)). In turn, the AGG consulted an external statistician to evaluate the statistical analyses presented in the report (see B.6.5.12.1 (CA 5.5-017)) and to re-perform some key analysis with the Peto method based on the original study data. Overall the study is concluded to be acceptable.

The carcinogenic potential of glyphosate technical was assessed in an 18-month feeding study in male and female Swiss albino mice. Groups of 50 mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm glyphosate technical (equal to an intake of 0, 14.5, 149.7 and 1454 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females). Observations covered survival, clinical signs, neurological changes, body weight, food- and water consumption, ophthalmological examinations, masses formation, blood smears with differential count analysis, organ weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues and lesions for all control and high dosage group animals died, sacrificed moribund or killed at termination.

The survival after 18-month of treatment was 56, 60, 56 and 46 % in males and 68, 68, 60 and 60 % in females in the control through high dosage groups, respectively. The mortality (combined for both sexes) was slightly increased at the high dose level with 38, 36, 42 and 47 % for the control, low, mid- and high-dose group, respectively. There were no treatment-related effects on clinical signs, behaviour, eyes, body weight, body weight gain, food consumption, differential white blood cell counts in both sexes, gross pathology or organ weight data.

Degenerative changes in the heart were noted in high dose males, however, as the increase was not statistically significant and within HCD range, this findings was considered incidental. The number of malignant lymphoma was slightly elevated in the high dose group compared to control.

The RMS proposes a systemic NOAEL of 10000 ppm (equal to 1454 and 1467 mg/kg bw/day in males and females, respectively), the highest dose tested.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	Glyphosate
Description:	Solid white, odourless crystals
Lot/Batch #:	01/06/97
Purity:	> 95% (w/w)
Stability of test compound:	Expiry: December 1999

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	Swiss albino, HsdOla: MF1
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	Males: 25 – 47 g, females: 21 – 26 g
Acclimation period:	5 days
Diet/Food:	Ssniff rat/mouse powder food maintenance meal – low in germs (M/s Ssniff Spezialdiäten, D-59494 Soest, Germany), *ad libitum*
Water:	Well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	In groups of five per sex in polypropylene mouse cages with stainless steel top grill and steam sterilised clean paddy husk bedding.
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 30 - 70 % Air changes: 12 - 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1997-12-23 to 1999-06-29

Animal assignment and treatment:
In a carcinogenicity feeding study groups of 50 Swiss albino mice per sex received daily dietary doses of 0, 100, 1000 and 10000 ppm (equivalent to mean achieved dose levels of 0, 14.5, 149.7 and 1454 mg/kg bw/day for males, and 0, 15.0, 151.2 and 1466.8 mg/kg bw/day for females) glyphosate technical in diet for 18 month. The dose levels were chosen based on results of a 50-day pre-study in mice. Test diets were prepared prior to start of treatment and then in intervals ranging from 10 to 23 days. Diets were prepared in quantities of 10, 12 or 15 kg. For preparation of 12 kg diet mixtures 1.2 g, 12 g and 120 g for the low-, mid- and high-dose group, respectively,

of the test substance was mixed with approximately with 0.5 kg basal diet and blended for 3 minutes. This pre-mix was then mixed manually with approximately 0.5 kg food and then added in portions to the remaining bulk amount of food (approximately 11.0 kg) and blended in a stainless steel ribbon mixer for 20 minutes.

The homogeneity of the test material in diet was determined at beginning of treatment, and at 12 and 18 month. Analyses for achieved concentration were done at three and six month of the study. The stability of glyphosate technical in the diet was determined prior to start of the study for the 100 and 10000 ppm dose levels.

Clinical observations
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. For mice with observed tumours a separate record was maintained with details of the tumour development.

Body weight
Individual body weights were recorded on Day 1 (prior to treatment) and at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each cage group from week 1 to week 13 and once a month thereafter. Food efficiency and compound intake was calculated from the recorded food consumption data.

Haematology
Blood smear samples were collected at 9 month and at termination (18 month) from all surviving animals, and from mice that were killed in extremis. Differential white cell counts were performed on all blood smear samples.

Ophthalmological examination
Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mydriasis was induced before examination by adding 1 % Tropicamide solution into the eyes. All other grossly visible eye findings were recorded also at the daily observations.

Sacrifice and pathology
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. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: adrenals, kidneys, liver and gall bladder, ovaries and testes.

Tissue samples were taken from each mice from the following organs and preserved in 10 % buffered neutral formalin: adrenals, bone & bone marrow (sternum and femur (incl. joint)), brain (incl. cerebrum, cerebellum pons), caecum, colon, duodenum, epididymides, eyes (with optic nerve), heart, jejunum, ilium, kidneys, larynx, liver and gall bladder, lungs, lymph nodes (mandibular, mesenteric, and superficial inguinal), mammary gland (female only), muscle (femoral), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles and coagulating glands, skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and all lesions and tumours/masses.
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, tissues of gross lesions and masses from all mice were examined microscopically.

Statistics
Body weight, body weight gain, food consumption and differential leukocyte counts of different groups were compared by Bartlett´s test for homogeneity of intra group variances. Heterogeneous data were transformed using log transformation. Data with homogeneous intra group variances were subjected to one-way analysis of variance using ANOVA. When “F” values were significant, Dunnetts pair wise comparison of means of treated groups with control means was done individually.

Incidence of gross lesions and non-neoplastic histopathological changes and incidences of benign and malignant neoplasms in the test substance groups were statistically compared with control group by Z-test where necessary. The incidence of neoplasms was analysed by Cochran-Armitage linear trend test, Life table analysis for fatal tumour incidence and Peto´s incidental tumour analysis. When a significant difference over the control group was observed in any of the treatment groups, the dose correlation co-efficient was estimated and subjected to t-test. All analyses and comparisons were evaluated at the 5 % level and statistically significant differences ($p \leq 0.05$) were indicated.

Note AGG : The statistical analysis conducted in the study report was not considered appropriate (see also deviations from current test guideline above). Therefore, re-evaluation of the study results was conducted reported in doc CA 5.5-017.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 8.37% at the 100 ppm level and 6.99% at the 10000 ppm level, when stored at room temperature in PE bags inside stainless steel drums.

Analyses for homogeneity at the start and at 12 and 18 month of treatment indicated that the dose preparations were homogeneous. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10 % of the nominal concentration for all diet samples. The overall mean achieved concentrations were 94.6 ± 2.3, 945.5 ± 23.7 and 9410 ± 191 as compared to the nominal concentrations of 100, 1000 and 10000 ppm, respectively at the start of the study and 91.87 ± 2.92, 958.0 ± 34.2 and 9495 ± 216 at 18 months.

B. MORTALITY

The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in the table below.

Table B.6.5.11-1: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Cumulated mortalities after 78-week dietary exposure to glyphosate technical

Sex	Historical control[#]		Dose group (ppm)**			
	min- max[*]	Mean ± SD	0	100	1000	10000
Male	11/50 – 27/50	18 ± 5	22 (6)	20 (6)	22 (8)	27 (8)
Female	12/50 – 20/50	16 ± 3	16 (7)	16 (7)	20 (2)	20 (3)
Combined sex	12/100 – 47/100	17 ± 4	38 (13)	36 (13)	42 (10)	47 (11)

[#] Derived from the control groups of 9 studies performed in the timeframe embracing the study summarised here
* Number of dead animals / total number of animals
** Total number of animals per group = 50
() number of animals killed in extremis

The percentage of survival in each of the dose groups are summarised below.

Table B.6.5.11-2: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Percentage survival at termination after 18-month dietary exposure to glyphosate technical

Sex	Dose group (ppm)			
	0	100	1000	10000
Male	56	60	56	46
Female	68	68	60	60
Combined	62	64	58	53

The survival percentage was slightly decreased at the high dose level, but the decrease did not attain statistical significance.

As can be seen from the historical control data, the mortality in the high-dose group is, at the upper end, but within the historical control range. The number of animals that were killed in extremis were comparable or higher in the controls compared to the treated groups.

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

D. BODY WEIGHT

There were no significant treatment-related effects on male and female body weight and overall body weight gain during the conduct of study.

In males incidences of slightly decreased body weights in week 10 at 100 ppm and in months 7 and 8 at 1000 ppm were considered incidental, since no effects on body weights were observed in the high-dose group.

In females receiving 100 ppm, decreased net body weight gain (85 % of control) was observed in month 18 only. At 1000 and 10000 ppm the body weight gain was 89 % and 99 % of the control. Therefore, this finding was also considered as incidental.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

The observed slightly lower food consumption observed in males in week 1 at 100 ppm and in weeks 1 and 7 at 10000 ppm was considered incidental, since the changes were minimal and the effects was not consistent during the remaining parts of the study period.

In females lower food consumptions were observed in week 2 for all dose levels, in week 26 at 10000 ppm. Higher food consumption occurred in week 11 at 100 ppm and in weeks 3 and 4 at 10000 ppm. These findings were also considered incidental, since the changes were minimal and food consumption during the remaining parts of the study was comparable with the control group.

The calculated mean daily test substance intake is summarised in the **Table** below.

Table B.6.5.11-3: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Group mean compound intake levels

Dose group	Dietary concentration (ppm)	Mean daily test substance intake (mg/kg bw/day)*	
		Males	Females
1 (control)	0	0.0	0.0
2 (low)	100	14.5	15.0
3 (mid)	1000	149.7	151.2
4 (high)	10000	1453.8	1466.8

* based on actual food intake and body weight data

F. HAEMATOLOGY

Differential leukocyte counts at 9 and 18 month

There were no significance treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophil counts (+2.5%) and lymphocyte counts (7.7%). in high dose males at 9 month. Since the effect was only slight and no statistically significant effect occurred at 18 months it is considered to be incidental.

The higher eosinophil counts at 9 months, higher neutrophil and monocyte counts at 18 months, as well as slightly lower lymphocyte counts observed in high dose females at 18 month at 18 months were not consistently observed, only marginal and therefore considered incidental.

Differential leukocyte counts of moribund sacrificed mice
Although the differential leukocyte count data were not statistically analysed, it does not appear that a dose response related effect occurred.

G. OPHTHALMOLOGICAL EXAMINATION

There were no treatment-related findings observed at the ophthalmological examinations performed at 6, 12 and 18 month of treatment.

H. NECROPSY

Gross pathology
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.

In animals found dead or sacrificed moribund across control and all dose levels the incidence of enlargement of superficial inguinal lymph nodes and thymus in mid dose females and in the high dose for combined sexes was statistically significant increased. These enlargements were associated with neoplasms of the hemolymphoreticular system. Other changes included enlargement of various lymph nodes, and thymus, both associated with neoplasms of the hemolymphoreticular system, enlargement of the spleen, associated with neoplasia and amyloidosis and increased extramedullary haematopoiesis. The low incidence of observed liver enlargements was associated with neoplasia and amyloidosis. However, none of these findings were dose-dependent.

Table B.6.5.11-4: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Gross pathology finding in animals found dead and moribund

Incidence	Males				Females				Combined sex			
	C	L	M	H	C	L	M	H	C	L	M	H
No. of mice examined	22	20	22	27	16	16	20	20	38	36	42	47
Lymph nodes-Superficial. Ing.L.Node							+					
- Enlarged	7	5	5	6	2	6	9	4	9	11	14	10
Liver												
- Enlarged	3	1	3	1	3	4	2	2	6	5	5	3
Mandibular lymph nodes												
- Enlarged	3	6	3	3	1	2	3	3	4	8	6	6
Mesenteric lymph nodes												
- Enlarged	8	9	8	11	8	9	10	7	16	18	18	18
Skin												
- Alopecia/Patchy alopecia	2	1	0	3	1	0	2	0	3	1	2	3
Spleen												
- Enlarged	8	8	11	13	10	11	12	7	18	19	23	20
Thymus										+		+
- Enlarged	0	1	0	3	0	3	1	2	0	4	1	5

C: Control L: Low dose M: Mid dose H: High dose
+/-: Significantly higher(+)/lower(-) than the control group

In mice sacrificed at termination the following changes were observed: Kidney surface rough/uneven in high dose males, discoloration / enlargement of mesenteric lymph nodes in high dose females and discoloration in high dose combined sex, and enlargement of spleens in both sexes combined at the high dose were significantly higher than in control mice. The study author did not consider these findings to be treatment related arguing that none of these

changes showed a dose-dependency, and that the corresponding histopathological changes were not significantly higher in these groups,. The RMS does not agree with the lack of dose-response argument but does agree that no underlying increase in histopathological findings were observed.

Table B.6.5.11-5: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Gross pathology finding in animals found dead and moribund

Incidence	Males				Females				Combined sex			
	C	L	M	H	C	L	M	H	C	L	M	H
No. of mice examined	28	30	28	23	34	34	30	30	62	64	58	53
Kidneys				+								
- Surface-rough/uneven	1	2	2	5	0	0	0	0	1	2	2	5
Lymph nodes-Axillary				-								
- Enlarged	5	3	5	0	3	6	1	4	8	9	6	4
Lymph nodes- Superficial. Ing.L.Node												
- Enlarged	3	1	2	2	0	3	1	2	3	4	3	4
Liver												
- Mass(es)	4	4	2	2	1	1	2	2	5	5	4	4
Mandibular lymph nodes												
- Enlarged	12	19	19	14	15	15	12	13	27	34	31	27
Mesenteric lymph nodes								+				+
- Discolouration	7	9	7	6	2	3	2	12	9	12	9	18
								+				
- Enlarged	13	14	15	10	10	12	9	19	23	26	24	29
Ovaries												
- Bursal cyst	NA	NA	NA	NA	13	11	11	13	--	--	--	--
Spleen												+
- Enlarged	8	11	11	10	9	15	6	14	17	26	17	24

C: Control L: Low dose M: Mid dose H: High dose
+/-: Significantly higher(+)/lower(-) than the control group

Organ weights
There were no treatment-related findings observed in organ weights or relative organ weights.

Histopathology

Non-neoplastic changes:
Cystic glands of the stomach were significantly increased in high dose mice of both sexes combined.

There was a more frequent occurrence of cystic glands of the stomach in male mice. The effect becomes more evident when the incidences in animals found dead or moribund and those sacrificed at scheduled termination are combined. The difference between the treated groups is not large and, taking into account the large dose spacing, a clear dose response may be doubted but, according to the study author, the incidence was higher than the historical control data. Unfortunately, this historical control data was not presented in the report. In contrast, no increase became apparent in females.
Based on the incidences and the statistical significance mentioned for male animals, there was no NOEL in this study because it cannot be excluded that this finding was due to treatment. The clinical relevance of cystic glands of the stomach is not clear. In any case, there was no increase in severity (always minimal to mild) and, more important, the cysts formation did not progress to any other pathological lesion, even at a dose level that was 100 times higher than the lowest. Thus, this finding should not be taken into account when the NOAEL for this study is set.

Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex although in males without a dose-response.

In mandibular lymph nodes lymphoid hyperplasia was significantly increased 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.

Cell debris in tubules of epididymides was increased in mid dose males while the incidence of lymphocyte infiltration of the epididymides was decreased in mid-dose males. The incidence of sub-capsular cell hyperplasia was increased in adrenals of low dose males found dead and moribund sacrifice. In addition, the incidence of kidney nephropathy in mid-dose females was significant decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental.

Degenerative heart changes were higher in high-dose males, and significant higher in combined sex. The study report concludes that since the incidences within historical control data (refer to table B.6.5.11-6) that the effect was incidental. The RMS agrees with this conclusion.

Table B.6.5.11-6: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Summary of non-neoplastic histopathological findings, total incidence

	Dietary concentration of glyphosate (ppm)											
	Males				**Females**				**Combined sex**			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Stomach												
Number examined	50	50	50	50	50	16	20	50	100	66	70	100
Cystic glands (n)	17	27	31	33	23	4	5	25	40	31	36	58
Cystic glands, incidence [%]	34	54	62	66	46	25	25	50	40	47	51	58
Cystic glands, HCD [%], range (determined from 5 studies, dates 1996 to 1999)	0 - 45				0 - 35				0 - 39			
Bone (femur)												
Number examined	50	20	22	50	50	18	21	50	100	38	43	100
Haematopoiesis	2	1	8*	5	1	1	2	4	1	2	10	8
Mandibular Lymph node												
Number examined	50	48	49	50	50	48	48	49	100	96	97	99
Increased extramedullary haematopoiesis	8	9	14	12	4	10	8	6	12	19	22*	18
Lymphoid hyperplasia	19	28*	27*	21	24	27	25	17*	43	55	52	38
Spleen												
Number examined	50	50	49	50	50	49	47	50	100	99	96	100
Increased extramedullary haematopoiesis	13	20	12	8	14	23*	13	16	27	43	25	24
Epididymides												

Table B.6.5.11-6: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Summary of non-neoplastic histopathological findings, total incidence

Finding	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
	0	100	1000	10000	0	100	1000	10000	0	100	1000	10000
Number examined	50	21	22	50								
Cell debris in tubules	0	1	4	1								
Lymphocyte infiltration	4	1	0	4								
Adrenals												
Number examined	50	19	22	50	50	16	20	49	100	35	42	100
Sub-capsular cell hyperplasia	18	8	7	23	39	11	13	37	57	19	20	50
Kidney												
Number examined	50	26	26	50	50	18	21	50	100	44	47	100
Nephropathy	16	11	13	18	10	3	1*	5	26	14	24	23
Heart												
Number examined	50	22	22	50	50	16	20	50	100	38	42	100
Degenerative changes	25 (50%)	15	13	33 (66%)	6 (12%)	2	4	7 (14%)	31 (31%)	17	17	40* (40%)
Degenerative changes, HCD [%], mean and range (determined from 5 studies, dates 1996 to 1999)	14.4% [0-72%]				4.8% [0-24%]				9.6% [0-48%]			

* significant change

Neoplastic changes:

One renal tubule adenoma was observed in the mid dose (1/26) and two in the high dose group males (2/50). The relevance of this observation is further discussed in Volume 1.

RMS comments: An increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose (Table B.6.5.11-7). The incidence was statistically significantly elevated as compared to the actual control groups with the statistical analysis using the z-test conducted in this study. However, during the previous EU evaluation it was concluded that the z-test as performed in the study report is not appropriate and the statistical analysis was repeated using the recommended[1] Fisher's exact test as well as the Cochrane-Armitage trend test. In both cases, no statistical significance was observed (p value > 0.05). The RMS notes that the applicant provided a re-analysis of the Peto-analysis used in this study (refer to section B.6.5.12-1). As the re-analysis performed by the applicant is not agreed, the RMS provided an own Peto-analysis (refer to section B.6.5.12-2). The incidence of malignant lymphoma is above the mean values of the (relatively small) historical control data and, for males, outside the historical control range. The relevance of the observed increase in malignant lymphomas in the context of the classification and labelling of glyphosate is discussed in Volume 1.

Table B.6.5.11-7: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Incidences of malignant lymphoma and comparison with historical control data (7 studies conducted between 1996 and 2002)

	HCD*		Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	0	100	1000	10000	0	100	1000	10000
Dead & moribund										

[1] OECD Guidance Document 116 on the Conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Supporting Test Guidelines 451, 452 and 453

Table B.6.5.11-7: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Incidences of malignant lymphoma and comparison with historical control data (7 studies conducted between 1996 and 2002)

	HCD*		Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Number examined	143	122	22	20	22	27	16	16	20	20
Number affected	33	71	9	12	13	13	9	10	13	12
Percentage affected	23.1%	58.2%	41.0	↑**60.0+**	↑**59.0+**	48.0	56.0	63.0	65.0	60.0
Mean %	23.4%	56.5%	--	--	--	--	--	--	--	--
Range %	0-44%	0-100%	--	--	--	--	--	--	--	--
Terminal sacrifice										
Number examined	257	278	28	30	28	23	34	34	30	30
Number affected	30	61	1	3	3	↑6+	9	10	6	13
Percentage affected	11.7%	21.9%	3.6	10.0	10.7	↑**26.1+**	26.5	29.4	20.0	↑**43.3+**
Mean %	10.9%	22.0%	--	--	--	--	--	--	--	--
Range %	0-24%	0-43%	--	--	--	--	--	--	--	--
All fates										
Number examined	400	400	50	50	50	50	50	50	50	50
Number affected	63	132	10	15	16	**19**	18	20	19	25
Percentage affected	15.8%	33.0%	20.0	30.0	32.0	↑**38.0+**	36.0	40.0	38.0	↑**50.0+**
Mean %	15.8%	33.0%	--	--	--	--	--	--	--	--
Range %	6-30%	14-58%	--	--	--	--	--	--	--	--

+ significantly increased according to the study report using the z-test (statistical re-calculation for all fates not significant using the Fisher's Exact test (p-value of 0.077 for males and 0.225 for females) and no trend using the Cochran-Armitage trend test (p-value of 0.0655 for males and 0.068 for females)
-- not examined/determined
* historical control data (7 studies conducted between 1996 and 2002, same strain, same laboratory)

Upon re-analysis of the study data commissioned by AGG using the Peto method (1980), a significant trend was seen for mesenteric lymph node haemangioma for which only incidental tumours were observed (p-values one-sided 0.0032 and 0.004 for 2- and 5-strata, respectively; refer to B.6.5.12.2). In the HCD data provided by the applicant, no cases of haemangioma in the mesenteric lymph node (or in spleen or mandibular lymph node) of females were reported (7 studies conducted between 1996 and 2002). The relevance of the observed increase in haemangioma in the context of the classification and labelling of glyphosate is discussed in Volume 1.

Table B.6.5.11-8: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2001): Summary of mesenteric lymph node haemangioma and haemangiosarcoma, total incidence (added by RMS)

	Dietary concentration of glyphosate (ppm)							
	Males				Females			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Mesenteric lymph node								
Number examined	50	49	49	48	50	48	48	50
Haemangioma	1	0	0	1	1	0	0	4[a]
Haemangiosarcoma	0	0	1	0	0	0	0	0

[a] statistically significant trend using the Peto method for females (p-values one-sided 0.0032 and 0.004 for 2- and 5-strata, respectively; refer to B.6.5.12.2)

Added by RMS:
It the previous RAR (2015) it was indicated that tumours of the hemophoreticular system are one of the most common tumours in mice accounting for the highest percentage of spontaneous tumours in this species. To support this claims additional data from literature on the background incidence of this tumour in mice in general and in Swiss mice in particular was provided in the previous RAR. This information is copied below. Since these public literature studies were not submitted in the current dossier the following statement could not be checked by the current RMS.

"In an article going back to the 1960ies, Swiss mice were considered to be prone to the induction of malignant lymphoma by leukemogenic agents such as 7,12-dimethylbenz(α)anthracen (Toth et al., 1963, ASB2015-2536). In control animals which apparently survived for up to 70 – 80 weeks, the incidence of malignant lymphoma was 13.6% in males and 10.5% in females but the number of animals per group was low. In another, similar experiment, the incidence in males was lower (5.5%) but, this time, accounted for 36.3% in females. This latter information may be considered the first published evidence of a remarkable sex difference in the frequency of this tumour type and a higher vulnerability of female mice as it was nearly consistently reported thereafter.
More than 10 years later, Sher (1974, Z22020) published a review on spontaneous tumour incidences in various non-inbred mouse strains, based on scientific articles that had been released between 1960 and 1974. For Swiss random-bred strains, lymphomas and leukemias were mentioned to occur as the most common tumours. However, again, extremely variable incidences ranging from 0 to 21.4% were reported in long term studies for untreated males, depending on strain and source. In female Swiss mice, the incidences varied even between 0 and 36.4%. The maximum incidence had been noted in minimally inbred Carworth CF-1 mice (not related to Swiss mouse strains) with 53% in females.
Roe and Tucker (1974, ASB2015-2534) reported an incidence of 22.5 to 27.5% of (not further specified) lymphoreticular neoplasms in male Swiss mice (n=80) if fed ad libitum but a much lower tumour rate when diet was restricted.
Tucker (1979, Z83266) found 18% of male Swiss albino mice (Alderley Part strain) and 28% of the females with lymphoma, nearly all of them malignant. Her analysis was based on 50 males and 50 females fed ad libitum from weaning for their lifespan with the last, very few surviving animals killed after 3 years.
A large colony of (minimally inbred)"Swiss-derived" Icr:Ha(ICR) mouse had a 15% incidence of lymphoma in total with an approximate 2:1 ratio between females and males (precise percentages not given). In addition, 5% of the mice had developed leukemia (Eaton et al., 1980, ASB2015-2537). Only lung tumours occurred more frequently (23%). With regard to Swiss mice in general, the authors emphasised that "... differences occur between colonies and even within a colony with the passage of time so that contradictory results may be obtained using 'Swiss' stock from different sources. For example, the incidence of spontaneous neoplasia, although seldom reported in detail, varies with source and age."
According to a more recent article (Taddesse-Heath et al., 2000, ASB2015-2535), a much higher incidence of hematopoietic neoplasia of 58% was observed in a colony of CFW Swiss mice in the USA. Lymphoma (mostly of B-cell origin) accounted for 85% of these cases giving a total incidence of nearly 50%. The authors ascribed these tumours mainly to "infectious expression of murine leukemia viruses". It is not known to which extent such a latent infection might have contributed to lymphoma incidences reported earlier or even in the studies described in this RAR. A possible etiologic role of oncogenic viruses had been suspected by Roe and Tucker (1974, ASB2015-2534) yet who complained that many scientists performing long-term studies would often ignore this problem."

Assessment and conclusion by applicant:
In conclusion, glyphosate technical was not carcinogenic in Swiss albino mice following continuous dietary exposure of up to 1460.3 mg/kg bw/day (average for both sexes) for 18 months. Based on, a more frequent occurrence of cystic glands of the stomach in male mice and an increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose mortality at the upper limit of the historical control range, the NOAEL in mice after chronic exposure to Glyphosate technical for 18 month is conservatively set at 1000 ppm, corresponding to 149.7 mg/kg bw/day for males, 151.2 mg/kg bw/day for females and 150.5 mg/kg bw/day for both sexes combined.

Assessment and conclusion by RMS:
Degenerative changes in the heart were noted in high dose males, however, as the increase was not statistically significant and withing HCD range, this findings was considered incidental. The systemic NOAEL was concluded to be 10000 ppm (equal to 1454 and 1467 mg/kg bw/day in males and females, respectively), the highest dose tested.

For the statistical analysis of the neoplastic findings, it is referred to re-analysis of the data (refer to B.6.5.12.2) The relevance of the neoplastic findings (malignant lymphomas and haemangioma in the mesenteric lymph node) is further discussed in Volume 1.

B.6.5.12. Long-term toxicity – mouse, statistical evaluation of pre-neoplastic and neoplastic lesions

B.6.5.12.1. *Re-analysis by [redacted] (2017) – submitted by the applicant*

Data point:	CA 5.5/017
Report author	[redacted]
Report year	2017
Report title	Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice
Report No	11921
Document No	90017583
Guidelines followed in study	No guideline followed
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Valid, Category 1 Conclusion AGG: unacceptable; the method used is considered technically correct, however, the statistical analysis was performed using incorrect tumour incidences. Refer to section B.6.5.12.2 where these differences are explained and where AGG has commissioned an external statistician to reperform the statistical analysis.

Full summary

This is a re-evaluation of the statistical analysis of histopathology findings recorded in the [redacted] Study No. TOXI: 1559.CARCI-M, CD-1, [redacted] 2001 (see CA 5.5/016), in which an increased incidence of malignant lymphoma was reported.

Groups of 50 male and 50 female Swiss albino HsdOla:MF1 mice were exposed to glyphosate technical (glyphosate acid) in the diet at 0 (control), 100, 1000 or 10000 ppm for 18 months. The incidences of non-neoplastic and neoplastic lesions were statistically analysed using the Z-test (pair-wise comparisons). The Cochran-Armitage linear trend test, Peto's incidental tumour analysis and life table analysis for fatal tumours were used for the statistical analysis of the neoplastic lesions.

The statistical evaluation performed within the report of [redacted] (2001) has to be considered inadequate and generally lacks clarity.

Non-neoplastic and neoplastic lesions were evaluated using the Z-test, which is inappropriate for the analysis of tumour incidence data (the Z-test assumes a normal distribution). Peto's incidental tumour analysis was performed without assigning a Peto code to the neoplasms, which is inappropriate for the application of this test. The Cochran-Armitage test was only applied to the tumour incidences in the high dose group and the control group, which is inappropriate for a trend test, where all dose levels should be considered.

To address these issues, the statistical significance of all tumour incidence data was re-assessed, starting from the raw data tables of the original report and applying appropriate statistical methods.

When appropriate statistics are applied to the tumour incidence data, (and the incidence data of malignant lymphoma in particular), no statistically significant increase in tumour incidence is found in this analysis.

Moreover, the study was compromised by the presence of non-identified ecto- and endoparasites in a large number of animals and non-identified micro-organisms in single animals. In the absence of any detailed information it must be considered that some of the findings in this study (e.g. dermal lesions) are induced by infectious agents.

All this taken together, it can be concluded that Glyphosate Technical (glyphosate acid) is not carcinogenic in Swiss albino mice when exposed to dietary concentrations of up to 10000 ppm for a duration of 18 months.

I. MATERIAL AND METHODS

Data entry and compilation

The animal heading data (animal number, sex, group, first and last day under test) were entered into the Pathdata System Version 6.2e2 (PDS Systems, Switzerland) at AnaPath GmbH, Switzerland, including:

- Groups
- Animal numbers and sex
- The first treatment day (according to study report, December 23, 1997) was entered for all animals.
- The last treatment day was entered according to the study report. In the study report, a list of decedents was provided. A list of interim decedent animals with the respective study day of necropsy is the basis for decedents (Table 10, study report pages 126-127). In this table, the study day of death was specified. The study day was calculated for the correct date. This date was entered as last day under study and as necropsy day for each decedent.
- For terminal sacrifices, no exact data were available. Therefore, the final day under study was calculated for these animals by the last day under treatment (June 29, 1999) (page 14 of study report) + a 14 days necropsy period (July 14, 1999).
- The mode of death (found death, sacrificed moribund, terminal sacrifice) was entered.

Thereafter the following data were entered:

- all neoplastic data
- all pre-neoplastic data insofar they are not related to inflammatory or infectious lesions
- all findings were entered as unilateral findings in bilateral organs
- All findings were entered as unilateral findings in bilateral organs.
- All neoplastic lesions were entered in the PathData system for statistical analysis. Systemic neoplasms (malignant lymphoma, myeloid leukemia and histiocytic sarcoma) were entered under ‘Systemic Neoplasms’. In the original report, they were entered under ‘Hemolymphoreticular System’. Systemic neoplasms were entered using pathology codes, i.e. ML for malignant lymphoma, MY for myeloid leukemia, HS for histiocytic sarcoma, along with a severity degree (scale 1 to 5). Non-inflammatory tissue infiltrates were given the code “M” for metastasis. A severity grade was not given for metastases because this was not available in the original study report.
- Systemic neoplasms that were originally described under ‘bone (femur and joint)’ or ‘sternum with bone marrow’ were separately entered for both organs in this report. Therefore, a ‘metastasis’ in these organs appears twice, for example for animal no. 1129, the original report states: ‘sternum with bone marrow: malignant lymphoma’. Under the re-evaluation, malignant lymphoma is entered as metastasis under sternum and bone marrow.
- Tumours were counted only once per animal, without any consideration of metastases.

Since no Peto codes were assigned to the primary neoplasms in the original study, the following Peto codes were assigned for statistical calculation:

- N0 (malignant neoplasm, no Peto code) in survivors
- N1 (incidental malignant neoplasm) was not applied
- N2 (probably incidental malignant neoplasm) in cases of systemic neoplasms in animals that survived and only one organ was affected
- N3 (probably fatal malignancy) in cases of systemic neoplasms or large squamous cell carcinomas in animals that were sacrificed moribund or survived the course of the study.
- N4 (fatal malignancy) in cases of systemic neoplasms or large squamous cell carcinomas or alveolar-bronchiolar carcinoma in animals that were found dead during the course of the study.
- B0 (benign neoplasm, no PETO code) in survivors

- B1 (incidental benign neoplasm) in Leydig cell tumours,
- B2 (probably incidental benign neoplasm) in cases of small hepatocellular adenoma, renal cell adenoma, adenoma in cecum, haemangioma in tail, and osteoma,
- B3 (probably fatal benign neoplasm) and B4 (fatal benign neoplasm) were not assigned.

The correctness of the transfer of the data from the original report to the PathData system was quality-checked. 100% of the data have been quality checked of which 20% under GLP by the local QAU. The microscopic diagnoses were entered directly into the PathData system.

An explanation of Codes and Symbols can be found at the beginning of the Tables section.

Following microscopic data are presented in the Tables:

- NUMBER OF ANIMALS WITH MICROSCOPIC LESIONS BY ORGAN/GROUP/SEX (incidences of non-neoplastic (pre-neoplastic) lesions without gradings)
- OVERALL CHRONOLOGICAL LISTING OF NEOPLASMS BY WEEKLY INTERVALS
- AOFT (Animal Organ Finding Table = table of individual microscopic findings)
- TEXT OF GROSS AND MICROSCOPIC FINDINGS (complete narrative of both the macroscopic and microscopic findings, including Animal Heading Data for each dose group).

Gross lesions were not entered since they are not relevant for the statistical evaluation of microscopical lesions.

Statistical re-evaluation

Neoplastic lesions
Statistical evaluation of all neoplastic lesions was carried out using the Fisher's Exact test and the test for positive trend with respect to dose rates according to Peto *et al*. (1980) with assignment of Peto codes to all neoplasms. For the Fisher's Exact test the one-tailed p-values are given for each pair-wise comparison and for the Peto test the trend and one-tailed p-values are given in the tables in annex to this report. When a neoplasm is marked with a "#", it is considered to be a common tumour.

Non-neoplastic lesions
Statistical evaluation of all non-neoplastic (pre-neoplastic) findings was carried out with the Fisher's Exact test and the trend test over all dose groups according to Armitage (1955), the Cochrane-Armitage linear trend test.

For the Fisher's Exact test the one-tailed p-values are given for each pair-wise comparison and for the Cochran Armitage test the trend and one-tailed p-values are given in the tables in annex to this report.

Assessment of statistical significance
For tumour incidences to be considered as statistically significant according to the "Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals" (CDER 2001) one-tailed p values should be less than 0.025 for rare neoplasms and less than 0.005 for common neoplasms (Haseman 1983, Lin and Rahman 1998). A neoplasm is regarded as rare, if in an assay involving one or two hundred animals there may be no such neoplasm, or at most one or two such neoplasms in animals of one sex and strain. Based on this definition of rare tumors, if only one or two animals have a particular type of neoplasm in a standard assay, a statistically significant result is not relevant. This holds true even if one or two such neoplasms occur in the top dose group and there are none elsewhere in the study. A neoplasm is regarded as common, "if it occurs spontaneously in five or ten or more animals in most experiments performed with animals of one strain" (Peto *et al*. 1980).

II. RESULTS

A: NEOPLASTIC LESIONS

No statistically significant trend was found for systemic neoplasms (including malignant lymphoma) in the Peto test. When the same tumours were analysed using the Fisher's Exact test, no statistically significantly increases were found in pair-wise comparisons of all dose groups with the control group.

Table 6.5.12.1-1: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
Number of animals		50	50	50	50	50	50	50	50
Cerebrum	Malignant lymphoma	2	-	2	1	-	2	4	2
Cerebellum	Malignant lymphoma	-	-	-	-	-	-	3	-
Medulla oblangata	Malignant lymphoma	-	-	-	-	-	-	2	-
Spinal chord	Malignant lymphoma	-	-	2	-	1	-	1	1
Sciatic nerve	Malignant lymphoma	-	-	1	1	-	2	1	-
Heart	Malignant lymphoma	4	-	3	1	5	4	4	4
Trachea	Malignant lymphoma	-	-	-	-	1	1	1	1
Lung	Malignant lymphoma	6	7	10	9	10	5	6	12
Systemic neoplasms	Malignant lymphoma	12	16	18	19	19	20	19	25
Oesophagus	Malignant lymphoma	-	-	1	-	-	-	-	-
Stomach	Malignant lymphoma	7	6	4	4	3	4	3	4
Duodenum	Malignant lymphoma	1	4	4	5	-	3	1	1
Jejunum	Malignant lymphoma	-	1	2	3	-	1	-	-
Ileum	Malignant lymphoma	4	3	4	8	2	7	2	5
Caecum	Malignant lymphoma	5	5	6	2	1	1	3	1
Colon	Malignant lymphoma	3	2	3	5	-	3	-	1
Rectum	Malignant lymphoma	4	2	2	5	2	4	3	3
Liver	Malignant lymphoma	7	9	10	14	9	6	10	10
Gallbladder	Malignant lymphoma	2	1	3	6	-	2	2	1
Pancreas	Malignant lymphoma	3	5	2	7	4	6	6	8
Kidneys	Malignant lymphoma	11	10	14	15	10	8	9	14
Urinary bladder	Malignant lymphoma	5	4	5	6	5	8	9	10
Ovaries	Malignant lymphoma	-	-	-	-	4	9	8	6
Uterus	Malignant lymphoma	-	-	-	-	1	8	7	7

Table 6.5.12.1-1: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	100	1000	10000	0	100	1000	10000
Testes	Malignant lymphoma	-	1	-	-	-	-	-	-
Epididymides	Malignant lymphoma	5	5	6	4	-	-	-	-
Prostate gland	Malignant lymphoma	3	4	-	4	-	-	-	-
Coagulating glands	Malignant lymphoma	1	3	2	4	-	-	-	-
Seminal vesicles	Malignant lymphoma	1	5	2	3	-	-	-	-
Pituitary gland	Malignant lymphoma	1	1	1	-	1	3	1	-
Thyroid gland	Malignant lymphoma	-	1	1	-	1	-	2	1
Parathyroid glands	Malignant lymphoma	-	-	-	-	-	1	1	1
Adrenal glands	Malignant lymphoma	2	4	3	4	4	6	4	5
Spleen	Malignant lymphoma	10	13	10	14	17	15	13	12
Bone marrow (sternum)	Malignant lymphoma	4	10	11	11	5	7	6	6
Bone marrow (smear)	Malignant lymphoma	2	6	5	5	1	3	5	2
Thymus	Malignant lymphoma	9	5	7	10	7	6	8	13
Inguinal lymph node	Malignant lymphoma	12	13	14	13	7	11	12	11
Other lymph node	Malignant lymphoma	1	5	5	3	8	8	6	4
Mesenteric lymph node	Malignant lymphoma	10	13	15	15	14	15	15	20
Mandibular lymph node	Malignant lymphoma	11	10	17	12	14	13	16	13
Salivary glands	Malignant lymphoma	7	5	9	6	9	5	6	10
Mammary glands	Malignant lymphoma	-	-	-	-	3	6	7	2
Skin/subcutis	Malignant lymphoma	-	2	2	2	1	-	3	-
Skeletal muscle	Malignant lymphoma	1	1	2	-	-	1	-	-
Mesentery	Malignant lymphoma	1	1	1	-	3	3	2	4
Joints	Malignant lymphoma	5	11	9	11	6	8	7	6
Eyes	Malignant lymphoma	2	2	4	4	2	1	2	2

Table 6.5.12.1-1: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2017): Selected neoplastic lesions (malignant neoplasms)

Dietary concentration of glyphosate (ppm)		Males				Females			
		0	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Optic nerve	Malignant lymphoma	2	2	4	4	2	1	2	2
Femur	Malignant lymphoma	6	11	9	11	6	7	7	6
Sternum	Malignant lymphoma	4	10	11	11	5	7	6	6
Body cavities	Malignant lymphoma	-	-	-	-	-	-	-	1

B: NON-NEOPLASTIC LESIONS

Non-neoplastic lesions (only pre-neoplastic lesions), including lymphoid hyperplasia's of lymphoid organs were analysed to determine possible early stages of systemic neoplasms such as malignant lymphoma. The only exceptions were the hyperplastic lesions in the skin. In most cases they were diagnosed as being associated with dermal inflammation or the presence of ectoparasites, which were not further specified. No statistically significant trend was found for any of the non-neoplastic lesions when the Armitage trend test was applied. Also, no statistically significant increases were evident in pair-wise comparisons with the control group using the Fisher's Exact test. The only exception was a statistically significant ($p < 0.05$) increase in the incidence of lymphoid hyperplasia in the inguinal lymph node ($p = 0.0269$) in high dose males which was not related to any neoplastic change.

Table 6.5.12.1-2: Statistical Evaluation of Pre-Neoplastic and Neoplastic Lesions from Study: Study No. TOXI: 1559.CARCI-M; Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice ([redacted] 2017): Selected non-neoplastic lesions

Dietary concentration of glyphosate (ppm)		**Males**				**Females**			
		0	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Number of animals		**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Inguinal lymph node	**Lymphoid hyperplasia**	4	8	1	4	1	1	**12***	8

* Statically significant from control (Fisher's exact test, $p \leq 0.05$)

Assessment and conclusion by applicant:

It can be concluded that Glyphosate Technical (glyphosate acid) is not carcinogenic in Swiss albino mice when exposed to dietary concentrations of up to 10000 ppm (equivalent to 1460.3 mg/kg bw/day) for a duration of 18 months.

Assessment and conclusion by RMS:

This re-analysis of the data from the carcinogenicity study by [redacted] (2001) was commissioned by the applicant. In order to check this re-analysis, the AGG consulted an external statistician to evaluate the statistical analyses presented in the report by [redacted] (2017) and to re-perform some key analysis with the Peto method based on the original study data.

The RMS conclusions are given at section B.6.5.12.2.

B.6.5.12.2. *Re-analysis by AGG*

Introduction

The applicant provided a re-analysis of the histopathology data of the study by [redacted] 2001 (B.6.5.11, CA 5.5/16, TOXI: 1559.CARCI-M) exposing Swiss albino mice to dietary glyphosate concentrations of up to 10,000 ppm for a duration of 18 months. In this study an increased incidence of malignant lymphoma This re-analysis performed by [redacted] 2017 (B.6.5.12.1, CA 5.5/17) was performed as the statistical evaluation performed within the report of [redacted] (2001) was considered inadequate by the study author. In the re-analysis no significant increase in the incidence of malignant lymphoma was found.

In turn, AGG has requested an external statistician to evaluate the statistical analyses presented in the report by [redacted] (2017) and to re-perform some key analysis with the Peto method. This analysis is summarized below by AGG and is based on the reports received in December 2020 and March 2021.

Box 1. Explanation on the Peto-test (1980) (added by RMS)

In the original study report ([redacted] 2001), a separate analysis of fatal and of incidental neoplasms was performed. The 1980 Peto-test is a way to combine the results of these two separate tests into a single test.

The reasons Peto introduced these tests, is that statistical analyses that only look at the total number of neoplasms per group could yield a distorted view when the intercurrent mortality differs between treatment groups. For instance, if in one of the groups half the animals die shortly after the start of the experiment, those animals will not have the opportunity to develop neoplasms. The number of neoplasms therefore is expected to be lower in this group than in groups with less mortality. Both the death rate method (which is the life table method applied in the original study report ([redacted] 2001)) and Peto's incidental tumour analysis are meant to take differences in intercurrent mortality into account.

Below the two separate tests (incidental tumour test and the death rate method) are explained and subsequently the combination in the 1980 Peto-test.

The first test is the Peto incidental tumour test. This test looks at the prevalence of a tumour in animals that died from another cause (i.e. the tumour is not considered fatal). That other cause may be another cause of death than the specific tumour or the terminal sacrifice (or other planned sacrifices). In order to take intercurrent mortality into account, the method uses age of death: this is because when animals die young, they will not have had the opportunity to develop the neoplasm. Therefore animals are compared only to other animals dying (or sacrificed) at a similar age. In order to do so, the age range is divided into categories, and the prevalence of the neoplasm is compared between groups within each age category. Thus, to carry out this method, age (or time-in-study) needs to be divided in classes.

The second method is the death rate method, which is applied to fatal neoplasms: neoplasms that are considered the cause of death of the animal. Such a death rate analysis was also carried out in the original study report ([redacted] 2001) although they referred to it as "life table analysis". For this analyses they only used the fatal neoplasms.

The Peto 1980 method simply combines the results from the two previous analyses. When both tests point in the same direction, the combined test will be some average, but might lead to a higher level of statistically significance than the individual tests. When analyses point in different directions, the combined test will yield a lower level of statistically significance.

When applying the tests explained above, it is essential that the observed neoplasms are divided in fatal neoplasms (causing the death of the animal) and incidental neoplasms (observed when the animal dies of other causes). As the Peto methods (1980) combines an analysis of incidental neoplasms with that of fatal neoplasms, p-values are calculated for three tests: fatal neoplasms (log rank test), incidental tumours and the combined test.

Short description of the carcinogenicity study

The carcinogenicity study ([redacted] 2001) used four dose groups: control (G1, 0 ppm), low (G2, 100 ppm), mid (G3, 1000 ppm) and high (G4, 10000 ppm), each of 50 males and 50 females. Histopathological examination was carried out on all tissues collected from the mice of the control and high dose groups; all pre-terminally dead and moribund sacrificed mice of the low and mid dose groups and all gross lesions of the terminally sacrificed mice from the low and mid dose groups. The tissues of the hemolymphoreticular system (i.e. spleen, mandibular and mesenteric lymph nodes) were also examined for both sexes of these groups, except where a diagnosis of malignant lymphoma had already been made on a tissue with a gross lesion.

Statistical methods used in the analyses of [redacted] 2001 and [redacted] 2017

In the original study report ([redacted] 2001) the data on pathology findings were analysed using a z-test to compare the number of neoplasms observed between groups, as well as the Cochran-Armitage linear trend test, Peto's incidental tumour analysis and a life table analysis. All statistical tests were evaluated at the 5% level. Although not explicitly stated, from the table this seem to have been two-sided tests.

In the [redacted] (2017) analysis three statistical methods were applied:
- A Fisher exact test to replace the z-test on neoplastic lesions
- A repeat of the Cochran-Armitage linear trend test on non-neoplastic lesions, as it was not clear from the original report whether the middle groups were included.
- The (1980) Peto-test combining incidental and fatal tumours was carried out, after assigning Peto codes to the tumours.

Furthermore, different levels of significance were applied by [redacted] (2017). For rare neoplasms, a one-sided p-value of 0.025 was applied, which yields the same statistical significance for rare neoplasms that increase with higher doses as the original study report ([redacted] 2001). However, for common neoplasms a much stricter criterion was applied: a one-side p-value of 0.005.

Comment on the statistical methods used by [redacted] (2017)

Use of Fisher's exact test instead of z-test
In the original report a z-test was carried out to compare proportions in two groups (identical to a chi-square test). As the expected numbers of neoplasms are often low, [redacted] is correct that Fisher's exact test is more appropriate. However, due to its exact nature, Fisher's exact test is conservative, which means that when it is used for testing, it yields statistically significant results less often than implied by the selected significance level.

Cochran-Armitage linear trend test
This test was carried out both in the original study report ([redacted] 2001) and in the re-analysis by [redacted] (2017). The trend test was repeated by [redacted] (2017) because [redacted] (2001) did not include the low and mid dose incidences when analysing the terminal sacrifice and all fates. [redacted] (2001) elected to do so because not all tissues in all animals were examined microscopically. Therefore they deemed inclusion of the low and mid dose groups in such analyses not valid. They did include the low and mid dose incidences in the analysis of outcomes where this was deemed appropriate. [redacted] 2017 ignores this validity argument and includes low and mid dose groups in all trend analyses, despite the fact that microscopical findings could have been missed in the low and mid dose groups as most of the tissues were not analysed for all animals.

If the dose-effect relation is linear, the Cochran-Armitage trend test will be more powerful than comparing the high dose to the control group. However, if the effect of exposure is mainly seen in the high dose group, this is not necessarily the case.

In carrying out trend tests, one has to assign a dose value to each category. Often the values 1, 2, 3 and 4 are used, but it is also possible to use other values, like the actual dose rate (0, 100, 1000 and 10000 ppm). From the output of the tables it can be inferred that the latter is done in the re-analysis by [redacted] (2017).

Given that [redacted] (2001) had a good reason not to carry out this trend test, the additional value of these tests by [redacted] (2017) are moot.

Peto test

Explanation of the test

In the original study report ([redacted] 2001), a separate analysis of fatal and of incidental neoplasms was performed. The 1980 Peto-test is a way to combine the results of these two separate tests into a single test.

The reasons Peto introduced these tests, is that statistical analyses that only look at the total number of neoplasms per group could yield a distorted view when the intercurrent mortality differs between treatment groups. For instance, if in one of the groups half the animals die shortly after the start of the experiment, those animals will not have the opportunity to develop neoplasms. The number of neoplasms therefore is expected to be lower in this group than in groups with less mortality. Both the death rate method (which is the life table method applied in the orginal study report ([redacted] 2001)) and Peto's incidental tumour analysis are meant to take differences in intercurrent mortality into account.

Below the two separate tests (incidental tumour test and the death rate method) are discussed and subsequently the combination in the 1980 Peto test.

The first test is the Peto incidental tumour test. This test looks at the prevalence of a tumour in animals that died from another cause (i.e. the tumour is not considered fatal). That other cause may be another cause of death than the specific tumour or the terminal sacrifice (or other planned sacrifices). In order to take intercurrent mortality into account, the method uses age of death: this is because when animals die young, they will not have had the opportunity to develop the neoplasm. Therefore animals are compared only to other animals dying (or sacrificed) at a similar age. In order to do so, the age range is divided into categories, and the prevalence of the neoplasm is compared between groups within each age category. Thus, to carry out this method, age (or time-in-study) needs to be divided in classes. Peto suggested a particular method to do so, but also observed that the exact way is not very important, as long as each class contains enough animals to enable each animal with an incidental tumour to contribute to the test, and as long as the classes are narrow enough to make the adjustment for intercurrent mortality valid.

The second method is the death rate method, which is applied to fatal neoplasms: neoplasms that are considered the cause of death of the animal. Such a death rate analysis was also carried out in the original study report ([redacted] 2001) although they referred to it as "life table analysis" (table starting at page 101 of the study report). For this analyses they only used the fatal neoplasms.

The Peto 1980 method simply combines the results from the two previous analyses. When both tests point in the same direction, the combined test will be some average, but might lead to a higher level of statistically significance than the individual tests. When analyses point in different directions, the combined test will yield a lower level of statistically significance.

When applying the tests explained above, it is essential that the observed neoplasms are divided in fatal neoplasms (causing the death of the animal) and incidental neoplasms (observed when the animal dies of other causes). Peto provides a system for coding the tumours (Peto codes). Table 6.5.12.2-1 shows the Peto codes assigned in the re-analysis by [redacted] (2017).

Table 6.5.12.2-1: Peto codes for tumour data as assigned by [redacted] (2017)

Peto code	N in Weber data	description
B0	54	Benign neoplasm in terminal sacrifice
B1	2	Benign neoplasm, definitely incidental
B2	14	Benign neoplasm, probably incidental
B3	2	Benign neoplasm, probably fatal
N0	68	Malignant neoplasm in terminal sacrifice
N2	6	Malignant neoplasm, probably incidental
N3	43	Malignant neoplasm, probably fatal
N4	77	Malignant neoplasm, definitely fatal

Comments on the application of the Peto method in original study report ([redacted] 2001) and re-analysis ([redacted] 2017)

Application of Peto method in original study report ([redacted] 2001)

In the report by [redacted] (2017) it is stated that in the original study report ([redacted] 2001) no Peto codes were assigned. However, in the original study report (page 50) it is stated that "*2. Life Table Analysis for Fatal Tumours: For compilation of incidence of fatal tumours required for Life table analysis for fatal tumours, the histopathological observations have been used as criteria for judgement of neoplasia as a cause of death or moribundity*" which implies that a Peto code was assigned. Also, the study report provides a list of animals whose moribundity was considered to be due to neoplasms (page 107/583).

However, in comparing the number of neoplasms in the incidental tumour analysis in Table 10.g (page 97) of the original study report ([redacted] 2001) with the numbers in Table 22 (page 222), it seems that in the original analysis by [redacted] also the fatal neoplasms were included as incidental tumours analysis. This makes this analysis more into a kind of overall analysis (of both incidental and fatal tumours). In failing to exclude the fatal neoplasms from the incidental neoplasms analysis, this analysis becomes a crude version of the combined Peto test. In this "crude version" the adjustment for intercurrent mortality for fatal neoplasms is less accurate than in the combined Peto test.
For the incidental tumour analysis, [redacted] (2001) used cut-off points of 300, 400 and 500 days making categories ("0-300, 301-400, 401-500 and 501-terminal sacrifice). This does not completely agree with the recommendations of Peto et al. (1980) who advised to treat planned sacrifices as a separate category, as the necropsies might be carried out slightly different for terminal sacrifices. However, this probably does not make a lot of difference to the outcomes.

Application of Peto method in the re-analysis by [redacted] (2017)

Assignments of Peto codes in [redacted] (2017)

As described above, essential for the Peto method is that tumours are characterized as either fatal (the animal died because of the tumour) or incidental (discovered in animals that died of other causes). For this, animals are assigned a Peto code (see Table 6.5.12.2-1 above), and in first instance tumours with codes N0-N2 and B0-B2 are taken together as incidental tumours, and codes N3-N4 and B3-B4 as fatal tumours. In a sensitivity analysis other divisions can be made (e.g. including N2 in the fatal tumours). [redacted] (2017) describes the way of assigning of Peto codes at page 16 of the study report, but this description is not completely in agreement with what was done. For instance, page 16 states that code B3 was not assigned, while this was assigned to two animals (Ma1467 and Ma1494; both LUNG Bronchio—alv. adenoma). In the re-analysis of the data upon request by AGG (refer to sections below), these were treated as fatal neoplasms (in line with [redacted] 2017), despite being benign. Furthermore, it is stated that code N3 could also be assigned to survivors, which is incorrect, as all tumours in terminal sacrifices should be classified as incidental tumours. However this was stated in the re-analysis report, but not applied as all terminal sacrifices were assigned codes N0 or B0. For systemic malignant lymphoma, four animals (Ma1217, Ma1275, Ma1386 and Ma1426) died before the terminal sacrifice and were assigned code N2 (probably incidental). On page 16 of the re-analyses report ([redacted] 2017) it is stated that code N2 was assigned "in cases of systemic neoplasms in animals that survived and only one organ was affected". According to appendix

40 of the original study report (2001), all four animals had malignant lymphoma in multiple organs, and did not survive, so it is not clear why this N2 code was assigned.
In this re-analysis, the analysis by is repeated. For the incidental tumours, age classes have to be made. In the re-analysis by (2017) in the report the output states that "ad hoc run time intervals" were used.

Output of the Peto analysis in (2017)

In the re-analysis by (2017), output is shown for the combined Peto test (starting at page 45 of the study report). It shows a trend and a p-value. The magnitude of the trend suggests it is the numerator of the test statistic, using 0, 100, 1000 and 10000 as dose values. However, given that the age intervals used in the re-analysis by are not known, the exact values could not be reproduced using the formulae used in Peto et al. 1980.

In the Peto method, a dose has to be assigned to each category. The output suggests that doses of 0, 100, 1000 and 10000 were used in the re-analysis by (2017) instead of using 1,2,3 and 4 (which is also commonly done). Assigning 0, 100, 1000, 10000 will put more emphasis on the findings in the high dose group than assigning 1,2,3 and 4, which seems reasonable choice.

Analyses were done separately on males and females by (2017).

Reperforming the key analyses with the Peto method

In order to better understand the analysis carried out in the re-analysis by (2017) and to validate its results, the application of the Peto method to the data was repeated. The results are presented below.

Data processing for repeat analysis

From the report of (2017), the raw data shown in the tables on raw data for the Peto analyse (starting on page 34) and the individual animal data for all 4 dose groups (starting on pages 225, 321, 423, 523) were copied, read in and used to check some of the calculations.

AGG supplied an Excel spreadsheet with pathology findings by dose group based on the findings of the original study report (2001), in which they reclassified some animals compared to the (2017) re-analysis. This spreadsheet included the identifiers of the animals in which each pathology was observed. In the analyses on the combined data this reclassification was applied to the data. The following animals were reclassified in the spreadsheet in comparison with the raw data (page 34 and on in the study report by (2017)):
• Animals MA1151, MA1162, MA1337, MA1195, MA1227 and MA1243: In the (2017) report those were listed as systemic malignant lymphoma, but not in the Excel spreadsheet by AGG;
• Animal MA1165: listed as myeloid leukaemia in the report, but as histoid by AGG;
• Animal MA1466: Listed as liver hepatocellular adenoma by AGG but not in the report;
• Animals MA1221, MA1298 and MA1318: Listed as kidney renal cell adenoma by AGG but not in the report;
• Animal MA1512: Listed as mesenteric lymph node haemangioma by AGG but not in the report;
• Animals MA1377 and MA1297: Listed as femur osteoma by AGG but not in the report.

The numbers of animals with systemic malignant lymphoma in the AGG spreadsheet equals that used in the original study report by (2001). The total number of systemic malignant lymphomas is 142, whereas according to the re-analysis by (2017) the number of malignant lymphoma in the category systemic neoplasms is 148.

Assignments of Peto codes for reclassified neoplasms

In the re-analysis, the Peto codes as found in the report (page 34 and on) were used. However, based on the AGG spreadsheet some additional neoplasms were added for which new Peto codes were assigned. Of these, animal Ma1165 (listed as myeloid leukaemia in the (2017) report, but as histiocytic sarcoma in the spreadsheet), animal Ma1466 (listed as liver hepatocellular adenoma in the spreadsheet, but not in the (2017) report) and animal Ma1377 (listed as femur osteoma by the spreadsheet but not in the (2017) study report) were terminal sacrifices, so were assigned code N0 (Ma1165) and B0 (Ma1466, Ma1377).

Animal Ma1512 was listed as mesentery lymph node haemangioma in the spreadsheet but not listed by [redacted]. This animal died of a neoplasm. This neoplasm was assigned B2 as this code was assigned by [redacted] in other animals with the same neoplasm.

Animals Ma1221, Ma1298, Ma1318 were listed as kidney renal cell adenoma by AGG but not in the [redacted] (2017) study report; However, as this category contained less than five neoplasms, it was not further analysed and therefore not added to the data file.

Animal Ma1297 was listed as femur osteoma in the spreadsheet but not in the [redacted] (2017) study report. This animal died of a neoplasm, but as the osteoma was judged to be benign, code B2 was assigned.

Together, four neoplasms were added and in one animal the type of neoplasm was changed.

Neoplasms removed from the analysis

In the [redacted] (2017) study report animals Ma1151, Ma1162, Ma1337, Ma1195, Ma1227, Ma1243 were listed with systemic malignant lymphoma, but not in the AGG spreadsheet (and thus not in the original study report). These neoplasms were removed from the dataset.

Furthermore, some records were present twice in the table starting at page 34 of the [redacted] (2017) study report. The second copy of these duplicate records were removed from the dataset.

Age classes used for the incidental tumour analysis

For the incidental tumor analysis, age classes have to be made. Peto et al. 1980 suggest making categories with increasing prevalence. For systemic malignant lymphoma this results in cutoffs for time at 448, 496 and 569 days. Given the small number animals with incidental tumours before the terminal sacrifice, this stratification is rather fine. Therefore also a second stratification in only two strata was applied: terminal sacrifice, and all earlier ages, as well as no stratification at all. These other stratifications were used as a sensitivity analysis, in order to see how sensitive the analysis is to the choice of stratification. In the AGG re-analysis, only the 5-strata and 2-strata versions, of which the 5-strata is the best, in the sense that it takes the age of the animals most accurately into account. The danger of a too fine stratification is that when a stratum is so small that only a few animals are included and that they could all be in the same dose group. In that case no statistical test can be conducted for that stratum, and the animals in that stratum are left out of the analysis. With the 5-strata used in the re-analysis this is not a problem: When performing the analysis separately for males and females, all strata have animals in all dose groups.

<u>Results of the re-analysis of the Peto method</u>

Initial data analysis

In order to determine how important it is to take intercurrent mortality into account, first the survival of the different dose groups is plotted below. This plot shows that the intercurrent mortality is higher in dose groups 3 and possibly 4 (mid and high) in females. Therefore these groups have less opportunities to develop neoplasms, and taking intercurrent mortality into account is expected to make the trend with dose stronger.

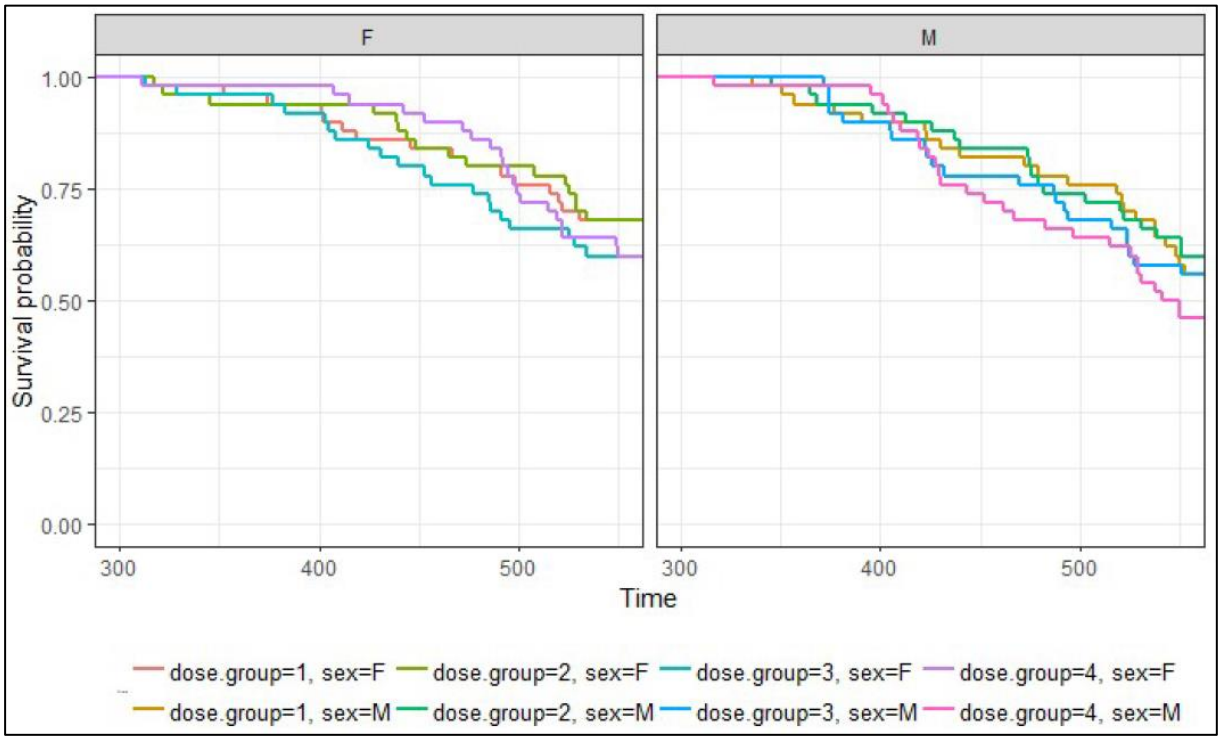


Figure 6.5.12.2-1. Survival over time for females (left) and males (right) in the four dose groups.

Application of the Peto (1980) method

The unit of analysis in the [redacted] (2017) study report are organ-specific neoplasms. That is, haemangioma in the tail is analysed as a separate neoplasm from haemangioma in mesenteric lymph node or in body cavities. In total, the analysis by [redacted] (2017) uses 31 neoplasms/organ combinations (see table 2) in the Peto-analyses described in [redacted] 2017 starting at page 34. Of these, after making the changes to the data described above, 16 neoplasms/organ combinations were only observed once, three only twice, and four only 3 or 4 times. Doing a complex stratified analyses on such low numbers is rather overkill. Therefore, the re-analysis of the data was only performed for the eight neoplasm/organ combinations with an incidence of at least five neoplasms. These are femur osteoma, liver hepatocellular adenoma, lung bronchioalveolar carcinoma, mesenteric lymph node haemangioma, systemic histiocytic sarcoma, systemic malignant lymphoma, systemic myeloid leukaemia and uterus stromal sarcoma.

Table 6.5.12.2-2: Neoplasm/organ combinations as analysed by [redacted] 2017 with the Peto-method, with the total number of neoplasms observed based on the original study report (changes are described above)

Neoplasm	# females	# males
FEMUR Osteoma	7	2
LIVER Hepatocell.adenoma	7	15
LUNG Bronchio—alv.adenoma	6	3
MESENT. LYMPH NODE Hemangiona	5	2
SYSTEMIC NEOPLASMS Histiocytic sarcoma	5	4
SYSTEMIC NEOPLASMS Malignant lymphoma	82	60
SYSTEMIC NEOPLASMS Myeloid leukemia	6	6
UTERUS Stromal sarcoma	8	0

As the Peto methods (1980) combines an analysis of incidental neoplasms with that of fatal neoplasms, p-values are calculated for three tests: fatal neoplasms (log rank test), incidental tumours and the combined test. Software R version 4.02 was used, which contains the log rank test. The incidental tumour test was programmed as the

extended Mantel-Haenszel test (Mantel 1963). It was programmed in duplicate, following formulae taken from 1) Rothman and Boice, 1979 and 2) Breslow and Day, 1980 and tested on the test datasets given in those books. The Peto combined test was taken from Peto *et al*. (1980).

As dose values in these trend tests 0, 100, 1000, 10000 was used.

Redoing the analyses of [redacted] 2017 as close as possible

The first analysis repeats the analyses by [redacted] 2017 as close as possible. This means that the changes to the data described above were NOT applied, and analyses were done for males and females separately (refer to tables below).

Table 6.5.12.2-3a: Results of the re-analysis as close as possible to [redacted] (2017); MALES

neoplasm	N fatal tumors	test fatal tumors	N incidental tumors	test incidental tumors		combined Peto test		p value Weber
		p-value		no strata p-value	5 strata p-value	no strata p-value	5 strata p-value	
FEMUR Osteoma	0	-	1	0.75	0.74	-	-	0.74
LIVER Hepatocell.adenoma	0	-	15	0.7	0.6	-	-	0.60
LUNG Bronchio—alv.adenoma	0	-	3	0.4	0.35	-	-	0.37
MESENT. LYMPH NODE Hemangioma	0	-	2	0.23	0.17	-	-	0.17
SYSTEMIC NEOPLASMS Histiocytic sarcoma	3	0.38	0	-	-	-	-	0.38
SYSTEMIC NEOPLASMS Malignant lymphoma	50	0.44	15	0.021	0.0076	0.14	0.11	0.11
SYSTEMIC NEOPLASMS Myeloid leukemia	5	0.57	2	0.83	0.81	0.74	0.72	0.77
UTERUS Stromal sarcoma	0	-	0	-	-	-	-	-

Table 6.5.12.2-3b: Results of the re-analysis as close as possible to [redacted] (2017); FEMALES

neoplasm	N fatal tumors	test fatal tumors	N incidental tumors	test incidental tumors		combined Peto test		p value Weber
		p-value		no strata p-value	5 strata p-value	no strata p-value	5 strata p-value	
FEMUR Osteoma	0	-	6	0.077	0.068	-	-	0.09
LIVER Hepatocell.adenoma	0	-	6	0.33	0.29	-	-	0.29
LUNG Bronchio—alv.adenoma	2	0.18	4	0.11	0.073	0.065	0.044	0.08
MESENT. LYMPH NODE Hemangioma	0	-	4	0.012	0.0063	-	-	0.06
SYSTEMIC NEOPLASMS Histiocytic sarcoma	4	0.51	1	0.042	0.036	0.23	0.22	0.22
SYSTEMIC NEOPLASMS Malignant lymphoma	42	0.3	41	0.11	0.1	0.11	0.11	0.10
SYSTEMIC NEOPLASMS Myeloid leukemia	6	0.68	0	-	-	-	-	0.68
UTERUS Stromal sarcoma	3	0.84	5	0.59	0.61	0.79	0.8	0.80

In the tables above one-side p-values for increasing incidence are given. In the output table in the study report by [redacted] (2017), neoplasms with an decreasing incidence are tested for decrease, so the p-value in the original table of [redacted] (2017) is 1 minus the p-value shown in the table above. The tables show that for the fatal tumours our re-analysis results and that of [redacted] (2017) agree exactly. For the analysis of incidental tumours or combined analyses, the p-values are, with the exception of mesenteric lymph node haemangioma in females, similar. The most plausible reason for the different p-value for mesenteric lymph node haemangioma in females is that the age stratification used by [redacted] (2017) was so narrow, that one stratum only existed of animals in a single dose group. In that case that stratum does no longer contribute to the test. Indeed, when animal Ma1498 (one of the two non-surviving animals with this neoplasm) is left out of the analysis, a p-value is calculated close to that reported by [redacted] (2017). That the p-values for the incidental tumour analysis are not completely equal can either be due to the use of another stratification for age, or it might be that the software used by [redacted] 2017 implemented a different estimator for the variance that is part of the test statistic, as there exist variants of this test (Breslow & Day, 1980).

Re-analysis by Peto method using the corrected database

Below in tables 6.5.12.2-4a/b the analysis is given using the data as reclassified in the AGG spreadsheet and using the correction that Peto code N2 is considered fatal. As explained above, only neoplasms with 5 or more occurrences are included (refer to Table 6.5.12.2-2 for the incidences). As dose values in these trend tests 0, 100, 1000, 10000 was used.

Table 6.5.12.2-4a: Results of the re-analysis; MALES (one-sided p-values)

Neoplasm	N fatal tumors	test fatal tumors	N incidental tumors	test incidental tumors		combined Peto test		p value Weber
		p-value		2 strata p-value	5 strata p-value	2 strata p-value	5 strata p-value	
LIVER Hepatocell.adenoma	0	-	15	0.61	0.6	-	-	0.60
SYSTEMIC NEOPLASMS Malignant lymphoma	47	0.23	13	0.0083	0.0083	0.046	0.046	0.11
SYSTEMIC NEOPLASMS Myeloid leukemia	5	0.57	1	0.73	0.73	0.65	0.65	0.77

Table 6.5.12.2-4b: Results of the re-analysis; FEMALES (one-sided p-values)

neoplasm	N fatal tumors	test fatal tumors	N incidental tumors	test incidental tumors		combined Peto test		p value Weber
		p-value		2 strata p-value	5 strata p-value	2 strata p-value	5 strata p-value	
FEMUR Osteoma	0	-	7	0.12	0.12	-	-	0.09
LIVER Hepatocell.adenoma	0	-	7	0.36	0.36	-	-	0.29
LUNG Bronchio-alv.adenoma	2	0.18	4	0.11	0.073	0.063	0.044	0.08
MESENT. LYMPH NODE Hemangiona	0	-	5	0.0032	0.004	-	-	0.06
SYSTEMIC NEOPLASMS Histiocytic sarcoma	4	0.51	1	0.036	0.036	0.22	0.22	0.22
SYSTEMIC NEOPLASMS Malignant lymphoma	44	0.36	38	0.037	0.037	0.087	0.087	0.10
SYSTEMIC NEOPLASMS Myeloid leukemia	6	0.68	0	-	-	-	-	0.68
UTERUS Stromal sarcoma	4	0.89	4	0.42	0.42	0.77	0.77	0.80

In the above tables one-side p-values for increasing incidence are given. In the output table in [redacted] 2017, neoplasms with an decreasing incidence are tested for decrease, so in case of decreasing incidence, the p-value in the original table of [redacted] 2017 is 1 minus the p-value shown in the table above. For liver hepatocellular adenoma in males and for lung bronchioalveolar adenoma, systemic histiocytic sarcoma and systemic myeloid leukaemia and the uterus stromal sarcoma in females, the numbers were the same as in the original analysis of [redacted] In these cases, the p-values were the same in case of fatal tumours, but could differ somewhat when incidental tumours were present. The differences between the 2-strata and 5-strata results show that the p-value for the incidental tumour test, and thus also that of the Peto test are slightly sensitive to the stratification used. The stratification used in [redacted] 2017 was not reported. Also a too narrow stratification could lead to removing a neoplasm from the analysis, namely in the case that all animals in the stratum with that neoplasm are in the same dose group. The latter is not a problem with the 5-strata used in the re-analysis. Another reason that the p-values for the incidental tumour analyses are not completely equal could be that the software used by [redacted] 2017 implemented a different estimator for the variance that is part of the test statistic, as there exist different variants of this test (Breslow & Day, 1980). In most cases the differences between the reanalysis and the results of [redacted] 2017 were small, but we calculated a slightly smaller p-value for lung bronchioalveolar adenoma in females. For the tumours where different numbers were used, results changed the most for malignant lymphoma in males (p-value 0.046 instead of 0.11) and haemangioma of the mesenteric lymph node in females (0.004 instead of 0.06). The first finding is due to the larger number of fatal tumours in males (50 instead of 45) in the AGG spreadsheet, the second due to the use of 5 instead of 2 tumours in the analysis.

Discussion

The current re-analysis showed that the analysis by [redacted] (2017) seems to be carried out correctly in a technical sense, although the stratification used for the incidental tumour analysis might have been too narrow for some

neoplasms. We could not fully reproduce the results for incidental cancers as the age categories used were not described. However, the differences in conclusion in the [redacted] (2017) study report compared to the original study report by [redacted] 2001, seem to result mostly from use of a different criterion for what is called statistically significant. Further, it presents analyses on many subgroups of neoplasms, which were often only observed in a few animals.

An important difference between the two reports is that different significance levels are applied. In the original study report ([redacted] 2001), a two-sided p-value of 0.05 was applied, while the [redacted] (2017) analysis applies a one-sided p-value of 0.025 to rare tumours and a one-side p-value of 0.005 for common tumours. As systemic malignant lymphoma, for which effects are seen, are not rare, this means that a rather strict criterion is used. [redacted] 2017 refers to the paper of Lin and Rahman (1998) for justification of the p-values applied. Choosing an appropriate p-value cut-off value for testing is based on weighing the risk on false positive findings against the risk of false negative findings: A lower p-value criterion will lower false-positive rates, but at the same time increase false negative rates. The choice for a best p-value criterion is therefore context dependent. The context of the Lin and Rahman paper is pharmaceutical drug development. In case of pharmaceuticals, the risk of carcinogenicity applies to the same person (the patient) that also reaps the benefits of the drug. This is another context as carcinogenicity testing of substances that are applied as herbicides. The paper of Lin and Rahman furthermore does not consider the risk on false negative findings extensively. They only state: "It is generally believed that pharmaceutical compounds are relatively safe since they have to go through many screenings before they can be tested on animals and humans. An overall false positive rate between 20% and 25% […..] is thought as too high. In that reasoning, the false negative issue is not the main concern". The applied significance levels therefore are open to discussion.

As systemic malignant lymphoma, for which effects are seen, are not rare, this means that under the strict criterion used by [redacted] the increase in systemic malignant lymphoma is not considered statistically significant, although the incidental tumours are significant in males (one-sided p-value of 0.0083).

In the carcinogenicity study by [redacted] (2001), the procedure for detecting neoplasms differed between the high dose and control group on the one hand, and the low and mid dose groups on the other hand: in the first two groups all tissues of all animals were histologically examined, whereas in the low and mid dose groups only tissues of animals that died prematurely, tissues with lesions and some specific tissues were examined. This means that the observations are not completely comparable between those groups, as some tumour types might be overlooked in the low and mid dose groups. The original study took this into account by only analysing the difference between the high dose group and the controls. In the [redacted] analysis this aspect is ignored.

Conclusion

The re-analysis of [redacted] (2017) seems technically largely correct. This is further acerbated by using Fisher's exact test which is conservative. The results presented in the [redacted] (2017) study report are basically not very different from the results in the original presentation of [redacted] (2001).

Assessment and conclusion by RMS:

The RMS is in agreement with the above assessment of the re-analysis by [redacted] (2017; reported under B.6.5.12.1). The main conclusions are that the re-analysis of [redacted] (2017) could largely be reproduced and the results seem technically largely correct. However, several differences were identified in the scoring of the tumours by [redacted] (2017). As listed above at section 'Assignments of Peto codes for reclassified neoplasms' and 'Neoplasms removed from the analysis' for a few animals neoplasms were missing, for one animal the type of neoplasm was incorrectly scored and, moreover, several animals were listed with systemic malignant lymphoma, while not in the original study report. This resulted in more conservative p-values for systemic malignant neoplasms in the analysis by [redacted] (2017). Therefore, the analysis by [redacted] (2017) is not considered acceptable by the RMS.

The results presented in the [redacted] (2017) study report are basically not very different from the results in the original presentation of [redacted] (2001). However, using a more stringent criterion for statistical significance meant that in the [redacted] report there were less effects declared statistically significant.

The re-analysis using the Peto method (1980) with some adjustments to the data (in agreement with the original study report) as presented above in Table 6.5.12.2-4a/b. The analysis showed a statistical significant trend for

mesenteric lymph node haemangioma in females, for which only incidental tumours were observed (one-sided p-values of 0.0032 and 0.004 for 2- and 5-strata, respectively).

It is noted that the p-values given are for one-sided testing. This was done in order to directly compare the results with the analysis by [redacted] (2017), in which one-sided testing was applied with a level of significance of 0.025 (which is equivalent to two-sided testing with a standard level of significance of 0.05). As the latter is in line with the statistical analysis established in the study protocol, the RMS is of opinion that results of two-sided testing should be reported. Therefore, the p-values from the AGG Peto-analysis should be multiplied by a factor of 2 to compare the values with standard level of significance of 0.05.
The relevance of the finding on mesenteric lymph node haemangioma in females is further discussed in Volume 1 at the overall evaluation of the tumour types.

For the other tumour types no statistically significant trends were observed when looking at the combined Peto test for males and female separately for the selected tumour types. However, for males, a significant increase was seen in incidental (e.g. non-fatal) systemic malignant lymphomas (one sided p-value of 0.0083 for both 2- and 5-strata), whereas no trend was seen in the combined Peto-test. When looking at the output-data, it was observed that the systemic malignant lymphomas classified as incidental systemic malignant lymphomas were only reported in surviving animals (at the terminal sacrifice), and that all malignant lymphomas classified as fatal tumours were observed in animals that died during the study.

Overall no significant trend was observed together with the high variability in the background incidence the apparent increase in malignant lymphomas is not considered treatment-related. However, a significant increase was seen in males in 'incidental' systemic malignant lymphomas (one sided p-value of 0.0083). It should be noted that the term 'incidental' tumours is used here in a different way. It does not mean that the tumours occurred by chance, but that these are classified by the study pathologist as non-fatal tumours (refer to Box 1 in the summary above for an explanation). When looking at the output-data, it was observed that the systemic malignant lymphomas classified as incidental systemic malignant lymphomas were only reported in surviving animals (at the terminal sacrifice), and that all malignant lymphomas classified as fatal tumours were observed in animals that died during the study. This means that the observed trend relates to the incidences of systemic malignant lymphomas at terminal sacrifice. For animals that were terminally sacrificed, incidences of 1/28 (3.6%), 3/30 (10%), 3/28 (10.7%) and 6/23 (26.1%) were reported. Although the incidence at the top dose is slightly outside HCD-range (range 0-24%; based on 7 studies conducted between 1996 and 2002), the increase is not considered treatment-related due to the high variability in the background incidence as reflected by the historical control incidences ranging from 0% up to 24% among different studies. Also, the incidence in the concurrent control group is at the low end of the HCD range (3.6%) and below the mean of (10.9%). Therefore, as 1) no significant effect was seen when all animals (dead, moribund sacrificed and terminal sacrificed animals) were taken into account based on the Fisher's exact test, the Cochrane-Armitage trend test as well as the combined Peto-test and 2) considering the high variability in the background incidence of this tumour type, the observed increase in malignant lymphomas in the terminally sacrificed animals is not considered of relevance for classification and labelling.

The findings on systemic malignant lymphomas are further discussed in Volume 1 at the overall evaluation of the tumour types.

B.6.5.13. Long-term toxicity – mouse, study 3

Data point:	CA 5.5/018 CA 5.5/019
Report author	[redacted]
Report year	1997
Report title	HR-001: 18-Month Oral Oncogenicity Study in Mice
Report No	[redacted]94-0151
Document No	NA
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984

	OECD 451 (1981).
Deviations from current test guideline (OECD 451, 2018)	The following deviations were noted from the current OECD test guideline: - mortality was observed only once per day; - histopathological examination of the cervix and lacrimal glands were not performed. Mammary gland was only investigated for females.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicants: Valid, Category 2a Conclusion AGG : The minor deviations compared to OECD 451 and not considered to be impact the validity of the study and therefore the study is concluded to be acceptable.

In order to evaluate the oncogenic potential of glyphosate technical in mice, the test substance was administered to SPF ICR mice (Crj:CD-1) by incorporating it into a basal diet at a concentration of 0, 1600, 8000 or 40000 ppm (equal to 0, 165.0, 838.1 and 4348 mg/kg bw/day for males and 0, 153.2, 786.8 and 4116 mg/kg bw/day for females) for a period of 18 months (78 weeks). During the treatment period, all animals were observed for clinical signs and measured body weights as well as food consumption. At week 21, urinalysis was carried out on 20 males from all groups. Differential leukocytes 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, organ weight analysis was conducted on 10 males and 10 females which were served to the determination of differential leukocytes counts. All animals of both sexes were subjected to necropsy and histopathological examinations.

- 40000 ppm group: In clinical observations, the incidence of pale-coloured skin was increased in males. In addition, loose faeces were observed in all cages beginning at week 21 in males and at week 20 in females. Retarded growth was persistently observed during treatment period showing statistically significant differences in weight from week 16 to 36 in males and from week 6 to end of treatment in females. These changes were associated with depressed food consumption and food efficiency. At necropsy, the increased incidences of distention of caecum were noted in males and females at terminal kill and in all animals examined, which were consistent to increase in absolute and relative weights of the caecum. However, no abnormalities were recorded in the caecum histopathologically. In males a significant increase was noted for the overall incidence of anal prolapsed which was correspondent to erosion/ulcer of the anus in histopathology. An increase in malignant lymphoma was noted in males.

- 8000 ppm group: Retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9-24. No treatment related changes were seen in males.

- 1600 ppm group: There were no treatment related changes in either sex in any parameters.

The NOAEL was concluded to be 1600 ppm, equal to 165.0 mg/kg bw/day for males and 153.2 mg/kg bw/day for females.

I. MATERIAL AND METHODS

A: Materials

1. **Test material:** Glyphosate technical

Identification:	HR-001	
Description:	Solid crystals	
Lot/Batch #:	T-941209	T-950308
Purity:	97.56 %	94.61 %
Stability of test compound:	Not mentioned in the report	

2. **Vehicle and/ or positive control:** Diet

3. Test animals:

Species:	Mouse
Strain:	SPF ICR (Crj:CD-1)
Source:	[redacted]
Age:	5 weeks at dosing
Sex:	Males and females
Weight at receipt:	Males: 15 – 25 g, females: 14 – 23 g
Acclimation period:	9 days in males; 7 days in females
Diet/Food:	Certified diet MF Mash (Oriental Yeast Co., Ltd.), *ad libitum*
Water:	Filtered and sterilised water, *ad libitum*
Housing:	In groups of four per sex in aluminium cages with wire mesh floors
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 15% Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-02-21 to 1996-09-06

Animal assignment and treatment:
Groups of 50 males and 50 females Specific-Pathogen-Free (SPF) ICR (Crj:CD-1) mice received the test material by incorporating it into the basal diet at a level of 0, 1600, 8000 or 40000 ppm for a period of 18 months.

Clinical observations
All animals were conducted a cage-side observation daily for clinical signs and their deaths during the study. In addition, a detailed examination including palpation of the body was performed at least once a week. Moribund animals showing marked debility were euthanised by exsanguinations under deep ether anaesthesia and necropsied when an unfavourable prognosis was predicted. Dead animals were taken from the cage as soon as possible after discovery in order to minimise the loss of tissues by cannibalism and necropsied. Mortality was expressed as ratios of cumulative number of animals found dead or killed *in extremis* to effective number of animal group.

Body weight
Individual body weights were recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 76. Body weights were also measured at week 78, at the end of treatment, and used for calculation of relative organ weights. Group mean body weights were calculated at each measurement.

Food consumption and compound intake
Food consumption by each cage was recorded for a period of 3 or 4 consecutive days weekly during the first 13 weeks and every 4 weeks from week 16 to 76. Food efficiency and compound intake was calculated from the recorded food consumption data.

Urinalysis
Urinalysis was conducted on 20 males at week 21. Parameters investigated included, glucose, ketones, occult blood, pH, protein and urobilinogen.

Haematology
Blood smear samples were collected at week 52 and at termination (18 month) from all surviving animals, and from mice that were killed *in extremis*. Differential white cell counts were performed on all blood smear samples.

Sacrifice and pathology
All animals that died or were killed *in extremis* during the conduct of the study were necropsied immediately.
All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Any macroscopic findings were recorded.

The following organ weights were determined from 10 mice per sex per group: brain, adrenals, kidneys, spleen, liver and gall bladder and testes.

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, tissues of gross lesions and masses from all mice were examined microscopically. The following tissues were examined: brain, spinal cord, sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, bone with marrow, tibio-femoral joint, lymph nodes, heart, aorta, salivary glands, oesophagus, stomach, liver with gallblader, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus, vagina, Harderian glands, eyes, skeletal muscle, skin, mammary gland (females only), all gross lesions.

Statistics
Body weight, food consumption and organ weights were evaluated by Bartlett´s test for homogeneity of intra group variances. When group variances were homogenous, a parametric analysis of variance of a one way layout type was conducted to determine if any statistical differences exist among groups. When the analysis of variance was significant, Dunnett's or Scheffe's multiple comparison test was applied. When the group variance were heterogeneous, the data were evaluated by Kruskal-Wallis non-parametric analysis of variance. When significant Dunnett type mean rank test or Scheffe's type mean rank test was applied.

Mortality was assessed by a life table analysis.

Urinalysis were analysed by Mann-Whitney's U test to compare data between the treatment groups and the controls.

Mann-Whitney's U test was used to analyse difference of the differential leukocyte counts between the high dose groups and the controls. For comparison of the data from all groups, Dunnett's and Scheffe's multiple comparison test was applied. The data from males killed *in extremis* during the treatment were examined by Mann-Whitney's U test.

Fisher's exact probability test was used to analyse the data of clinical signs and incidences of gross lesions at necropsy and histopathological lesions.

Results

A. ANALYSIS OF DOSE FORMULATIONS
Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss of 8.37 %. Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portion of the mixer. The coefficient of variation for each test diet was within 5.2 % or less. The results indicated that homogeneity of the test substance in diet was satisfactory in each test diet.

In order to verify concentration of the test substance in test diets, every batch of test diet was analysed during the treatment period. Mean concentration of the test substance in test diet at a nominal level of 1600, 8000 or 40000 ppm was 1561 ± 86.7, 7790 ± 394.4 or 38783 ± 1655.0 ppm (mean ± standard deviation), respectively. The values were within 97-98 % of the target concentrations and satisfied the acceptable limit of concentration for test substance.

B. MORTALITY
No significant differences were noted for mortality between the treated groups and the respective control of either sex. Cumulative mortality of each group of either sex is shown in the following table:

Table B.6.5.13-1: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Final mortality at termination of treatment (%)

Dose group (ppm)	Male	Female
0	24/50 (48)	18/50 (36)
1600	16/50 (32)	14/50 (28)
8000	23/50 (46)	10/50 (20)

Table B.6.5.13-1: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Final mortality at termination of treatment (%)

Dose group (ppm)	Male	Female
40000	21/50 (42)	15/50 (30)

C. CLINICAL OBSERVATIONS

Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:

Table B.6.5.13-2: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in clinical signs

	Male				Female			
	Dose group (ppm)							
	0	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
Number of animals examined	50	50	50	50	50	50	50	50
Perinasal region: tactile hair loss	0	↑3	↑3	↑**6***	5	↑**13***	↑9	↑8
Anus: mass(es)	0	0	0	↑**8****	0	0	0	0
Integument :								
wound	22	↓16	↓20	↓**6***	3	↓0	↓0	↓0
Erosion/Ulcer	9	↓5	↑12	↓8	16	↓**4****	↓**1****	↓**2****
Swelling	16	↓6*	↑13	↓9	6	↓2	↓0	↓1
Mass(es)	15	↓13	↓13	↓10	13	↓11	↓9	↓**4***
Pale-coloured skin	2	↑3	↑6	↑**10***	4	↓2	↑6	↑6
Hair loss	11	↑12	↑**21***	↑12	22	↑23	↓18	↓14
Wetted fur	11	↓9	↓7	↓**4***	1	1	1	1

* $p < 0.05$

** $p<0.01$ (Fisher's exact probability test).

In the 40000 ppm group, males showed increased incidences of tactile hair loss, pale-colored skin, and mass(es) of anus and decreases of wound and wetted fur. In females of this group decreased incidences were observed in ulcer/erosion and mass(es) of skin. Although, in addition to these signs, loose stool was observed in the cages of both sexes beginning at week 21 in males and 20 in females, the group housing failed to identify which animal excreted the loose stool.

In the 8000 ppm group, males showed an increased incidence in hair loss of the skin and females represented decreases in ulcer/erosion and swelling of the skin.

In the 1600 ppm group, males showed a decrease in swelling of the skin and females represented an increase in tactile hair loss as well as a decrease in ulcer/erosion of the skin.

None of the observed effects seems to be dose-related.

D. BODY WEIGHT

In the 40000 ppm group, males and females showed retarded growth during the treatment manifesting significantly lowered weights at weeks 16 to 36 in males and at weeks 6 and thereafter in females compared to the respective control. At the end of treatment, mean average weights were 93% and 86% of the respective control in males and females, respectively. Body weight gain was reduced by 15% in males and 26% in females

In the 8000 ppm group, females showed significantly decreased weights at week 6 and weeks 9 to 24 compared to the control and the final mean average weight was 92% of the control at the end of the treatment, while growth rate in males was comparable to the control. Body weight gain was decreased by 13%.

In the 1600 ppm group, males and females showed similar growth curves to the controls during the treatment period.

Effects on the body weight were more important in females than in males. These effects were durable in the 40000 ppm female group whereas they were stopped at week 36 in the male group of the same treatment dose. Sporadic effects were observed in the 8000 ppm female group. No significant effects were seen in the 1600 ppm male and female groups.

Table B.6.5.13-3: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Body weights at week 1, 24, 52 and 78

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
Body weight [g], mean ± SD									
Week 1		31.9 ± 1.8	32.0 ± 1.9	32.3 ± 2.0	31.4 ± 1.7	25.8 ± 1.4	25.3 ± 1.6	25.5 ± 1.5	25.7 ± 1.6
Week 24		47.6 ± 5.7	48.2 ± 5.9	48.5 ± 5.3	**44.6 ± 4.3***	47.4 ± 7.1	44.6 ± 7.2	**42.8 ± 7.4***	**39.1 ± 4.7****
Week 52		49.7 ± 7.0	51.4 ± 7.2	50.3 ± 6.3	46.3 ± 5.7	51.8 ± 10.9	51.7 ± 9.0	50.8 ± 9.3	**45.4 ± 5.9****
Week 78		50.7 ± 6.4	52.8 ± 6.9	50.1 ± 5.7	47.3 ± 5.8	55.8 ± 7.8	53.2 ± 8.2	51.5 ± 9.1	**47.8 ± 6.4****
Body weight [%], mean									
Week 1		100	100	101	98	100	98	99	100
Week 24		100	101	102	94	100	94	90	82
Week 52		100	103	101	93	100	100	98	88
Week 78		100	104	99	93	100	95	92	86

E. FOOD CONSUMPTION AND COMPOUND INTAKE

In the 40000 ppm group, males showed significant depressions in food consumption at weeks 1 and 68, revealing an overall group mean food consumption at 94% of the control during the treatment period. Females in this group also showed significantly decreased food consumption at weeks 1, 4, 8, 12, 20, 28, 40, 48 and 68, revealing an overall group mean food consumption at 93% of the control during the treatment period.

In the 8000 ppm group, females showed significantly lowered food consumption at weeks 28, 40, and 68 compared to the control manifesting an overall group mean food consumption at 96 % of the control. Whereas, food consumption in males was comparable to the control during the treatment period.

No statistically significant effects was observed in the 1600 ppm group either in males or females.

The food consumption depressions were more important in female than in males. They were not time-related.

Overall average chemical intake in each treated group of either sex was calculated from food consumption and nominal concentration as shown in the following table:

Table B.6.5.13-4: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Calculated test substance intake in mg/kg bw/day

Dose level (ppm)	Dose level (mg/kg bw/day)	
	Male	Female
1600	165.0	153.2
8000	838.1	786.8
40000	4348	4116

F. URINALYSIS

Urinalysis revealed a decrease in pH in the mid and high dose group. In addition, a decrease in protein was observed. The decrease in urinary pH is likely due to the presence of glyphosate in urine.

Table B.6.5.13-5: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Urinalysis (males only)

	Dose group			
	0	**1600**	**8000**	**40000**
pH				
- 8.0	4	4	0	0
- 7.5	5	11	**1****	0
- 7.0	7	2	2	0
- 6.5	3	3	11	0
- 6.0	1		6	**18****
- 5.0				2
Protein				
- +	4	8	6	13
- ++	12	12	12	**7****
- +++	4		2	

G. HAEMATOLOGY

Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table B.6.5.13-6: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in haematology parameters

Parameter	Sex	Fate of animals[a]	Dose group (ppm)		
			1600	**8000**	**40000**
Lymphocytes	Males	ke	ND[b]	↑139%	↑ 172%
	Females	tk	↓ 92	↑131%	↑ [c]163%
Neutrophil (segmented)	Males	ke	ND	↓ 91%	↓ 81%

Numbers in the above table show values in the treated groups when the corresponding value in the control group is 100.
a: ke, killed *in extremis*; tk, terminal kill; b: ND, not determined; c: Dunnett's or Scheffe's multiple comparison test
↓↑ Mann-Whitney's U test

In the 40000 ppm group, males killed *in extremis* during the treatment period showed an increase of lymphocytes in differential leukocyte counts and a decrease of neutrophil (segmented form). In females of this group, differential count of lymphocytes was significantly increased at week 78.

There were no significant differences in differential leukocyte counts at other intervals of examination in the 40000 ppm group of both sexes, males killed *in extremis* in the 8000 ppm group, and females at week 78 in the 8000 and 1600 ppm groups compared to the controls. No significant treatment-related effects were conceived in morphology of the leukocytes.

H. NECROPSY

Gross pathology

Statistically significant changes in incidence of macroscopic lesions observed in the treated groups of either sex are shown in the Table B.6.5.13-7.

In the 40000 ppm group, males and females showed significant increases in incidence of distention of the cecum at terminal kill after 78 weeks of treatment. Significant increases in incidence of the lesion were also noted in all animals examined recording 28 % (14/50) in males and 36 % (18/50) in females. Distended cecum was filled with loose stool-like materials. In addition, males showed an increase in loss of tactile hair and a decrease of cyct(s) in the kidney in those necropsied at terminal kill, and an increase of swelling in the lymph nodes (mesenteric) and a

decrease of wound in the skin in those killed *in extremis* or found dead during the treatment period when compared to the controls. Among these, significant differences in incidence were also noted in all animals examined for increases in loss of tactile hair and swelling of the lymph nodes (mesenteric) and a decrease in wound in the skin. Moreover, significant differences in incidence were also noted in all animals examined for an increase in anal prolapse of the anus and decreases in atrophy of the testis, partial amputation of the auricle, and swelling of the skin. Females showed decreases in loss of tactile hair and cyst(s) of the uterus in those necropsied at terminal kill, and an increase in swelling of the lymph nodes (others) and a decrease in ulcer/erosion of the skin in those killed *in extremis* or found dead during the treatment period. Among these, significant differences in incidence were noted in all animals examined for decreases in cyst(s) of the uterus and ulcer/erosion of the skin. Moreover, significant differences in incidence were also noted in all animals examined for decreases in opacity of the eye and loss of hair of the skin.

In the 8000 ppm group, males showed increases in mass(es) of the lung and loss of hair of skin and a decrease in soiled fur on external genital region in those necropsied at terminal kill when compared to the control. An increased incidence was also noted in all animals examined for loss of hair of the skin. Females killed *in extremis* or found dead during the treatment period in this group showed an increase in swelling of the lymph nodes (others) and decreases in coarse surface of the kidney and loss of hair of the skin. Moreover, significant differences in incidence were noted in all animals for an increase in mass(es) of the lung and decreases in swelling of the lymph nodes (cervical) and spleen, pale in colour and coarse surface of the kidney, opacity of the eye, and ulcer/erosion of the skin.

In the 1600 ppm group, males showed decreased incidences in swelling of the spleen in those necropsied at terminal kill and in all animals examined and in swelling of the skin in all animals examined, while females disclosed a decreased incidence in swelling of the spleen in all animals examined.

Table B.6.5.13-7: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in macroscopic lesions

Sex	**Male**				**Female**			
Dose group (ppm)	**0**	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
External appearance: Loss of tactile hair	0	0	↑1	↑5*	4	↑8	↑8	↓0*
Soiled fur on external genital region	9	↓7	↓2*	↓6	0	0	0	0
Spleen: enlargement	5	↓1*	↓4	↓2	7	↓2	↓3	↓3
Lung: Mass(es)	4	↑12	↑11*	↑9	8	↓6	↑18	8
Cecum: Distention	0	0	0	↑11**	0	0	0	↑16**
Kidney: Cyst(s)	4	4	↓2	0*	2	↓0	↑4	↓1
Uterus: Cyst(s)	-	-	-	-	6	↓2	↓2	↓0*
Skin: Loss of hair	1	↑4	↑7*	↑6	8	↑11	↑16	↓5
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Lymph nodes (mesenteric): Enlargement	0	↑2	0	↑5*	1	↑2	1	↑4
Lymph nodes (others): Enlargement	5	↓2	↓4	↑9	0	↑3	↑4*	↑4*
Kidney: Coarse surface	4	↓2	↓1	↓1	6	↓3	↓0*	↓4
Skin: Loss of hair	5	↓4	↑7	↓4	11	↓5	↓2*	↓4
Wound	6	↓2	↓3	↓0*	0	0	0	0
Ulcer/Erosion	6	↓3	↓4	6	5	↓3	↓0	↓0
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
External appearance: Loss of tactile hair	0	0	↑1	↑6*	5	↑11	↑9	↓3
Lymph nodes (cervical): Enlargement	5	↓3	↑6	↑9	12	↓6	↓4*	↓7
Lymph nodes (mesenteric): Enlargement	0	↑2	0	↑6*	3	↓2	↓1	↑5
Spleen: Swelling	16	↓4**	↓12	↓14	17	↓8*	↓8*	↓10
Lung: Mass(es)	9	↑14	↑17	↑15	10	↓8	↑20*	↑11
Cecum: Distention	0	0	0	↑14**	0	0	0	↑18**
Anus: Anal prolapse	0	0	0	↑5*	0	0	0	0

Table B.6.5.13-7: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in macroscopic lesions

Sex	Male				Female			
Dose group (ppm)	**0**	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
Kidney: Pale in color	6	↓2	↓4	↓2	7	↓4	↓1*	↓4
Coarse surface	6	↓2	↓2	↓1	7	↓4	↓0**	↓5
Testis: Atrophy	5	↓2	5	↓0*	-	-	-	-
Uterus: Cyst(s)	-	-	-	-	6	↓2	↓2	↓0*
Eye: Opacity	1	1	↑5	↑2	5	↓1	↓0*	↓0*
Auricle: Partial amputation	6	↓2	↓1	0*	4	↓2	↓0	↓1
Skin: Loss of hair	6	↑8	↑14*	↑10	19	↓16	↓18	↓9*
Wound	9	↓3	↓3	↓1*	0	0	0	0
Ulcer/Erosion	7	↓4	↑9	↓6	8	↓3	↓1*	↓0**
Swelling	7	↓1*	↓3	↓1*	3	↓0	↓0	↓0

Tk: Terminal kill; Ke/fd: Killed *in extremis* or found dead; All: All animals examined;
(N=) Number of animals examined
* $p < 0.05$ (Fisher's exact probability test);
** $p<0.01$

Organ weights
In the 40000 ppm group, males and females showed significant increases in absolute and relative weights of the cecum. The percentages of the values to those of the respective control were 173% in males and 187% in females for absolute weight, respectively, and 174% and 212% for relative weight, respectively. In females, relative weight of the kidney was also increased significantly at a level of 111% of the control.

Histopathology

Non-neoplastic lesions
Statistically significant changes in incidence of non-neoplastic lesions observed in the treated groups of either sex are shown in the Table B.6.5.13-8.

In the 40000 ppm group, males showed significant decreases in incidence of amyloid deposition in the liver in all animals examined and cyst(s) in the kidney in those necropsied at terminal kill and in all animals examined, when compared to the control.
In these males, erosion/ulcer in the anus was observed in a total of 8 animals including 6 cases killed *in extremis* or found dead during the treatment period and 2 cases necropsied at terminal kill. There was even a large abscess in one case. Among these, regressive hyperplasia of mucous epithelium of the large intestine was seen in 2 cases with severe lesions in the anus. However, as the histopathological examinations were carried out only on the anus which were observed macroscopic lesions, the incidence of erosion/ulcer in the anus was not assessed by a statistical method.
In females of this group, statistical significant decreases in incidence were noted in all animals examined as follows; increase haematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), cyst(s) in the kidney, micro-granuloma in the liver, and amyloid deposition in the spleen, liver, thyroid, and parathyroid. Among these, significant decreases in incidence were also noted for micro-granuloma in the liver in those necropsied at terminal kill and plasma cell hyperplasia in the lymph nodes (cervical) and amyloid deposition in the spleen and liver in those killed *in extremis* or found dead during the treatment period.

In the 8000 ppm group, although males did not show any non-neoplastic lesions with statistically significant differences in incidence from the control, females disclosed significant decreases in incidence of proliferation of cartilageous tissue in the tibio-femoral joint in those necropsied at terminal kill, wound in the skin in those killed *in extremis* or found dead during the treatment period, and subcutaneous abscess in the skin in all animals examined. In addition, significant decreases in incidence, when compared to the control, were observed in all animals examined as follows; increase haematopoiesis in bone marrow (femur, sternum and vertebra), plasma cell hyperplasia in the lymph nodes (cervical), extramedullary haematopoiesis in the spleen, amyloid deposition in the spleen, small intestine, liver, kidney (glomerular amyloidosis), uterus, thyroid, and parathyroid, and cataract in the eye. Among these, the incidences of extramedullary haematopoiesis in the spleen in those necropsied at terminal

kill and amyloid deposition in the spleen, liver, thyroid, and parathyroid in those killed *in extremis* or found dead during the treatment period were also decreased significantly.

In the 1600 ppm group, males in all animals examined showed a significant increase in incidence of alveolar epithelial cell hyperplasia in the lung and decreases in incidence of myeloid cell aggregation in the lymph nodes (mesentery) and extramedullary haematopoiesis in the spleen. In females of this group, the incidences in all animals examined were decreased significantly in increased haematopoiesis in bone marrow (femur) and amyloid deposition in the spleen, small intestine, liver, uterus, thyroid, and parathyroid. Among these, significantly decreased incidences were also noted for increased haematopoiesis in bone marrow (femur) and amyloid deposition in the small intestine and thyroid in those killed *in extremis* or found dead during the treatment period.

Except for the erosion/ulcer in the anus in high dose males all changes showed either a lack of dose-response or are not considered toxicologically relevant as decreases in incidence were observed.

Table B.6.5.13-8: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in non-neoplastic lesions

Sex	**Male**				**Female**			
Dose group (ppm)	**0**	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Spleen: Increased extramedullary haematopoiesis	5	2	4	3	6	5	1*	4
Liver: Micro-granuloma	1	5	5	4	15	16	14	7*
Kidney: Cortical cyst(s)	9	6	9	0*	2	1	5	0
Tibio-femoral joint: Proliferation of cartilageous tissue	14	17	11	15	18	14	11*	15
Ke/fd (N=)	(24)	(16)	(23)	(21)	(18)	(14)	(10)	(15)
Bone marrow (femur): Increased haematopoiesis	6	3	7	6	7	1*	1	2
Lymph nodes (cervical): Plasma cell hyperplasia	6	1	5	4	5	3	0	0*
Spleen: Amyloid deposition	2	3	2	0	8	3	0*	1*
Small intestine: Amyloid deposition	1	1	1	0	5	0*	0	2
Liver: Amyloid deposition	3	3	2	0	10	3	0**	1**
Thyroid: Amyloid deposition	2	2	2	0	8	1*	0*	2
Parathyroid: Amyloid deposition	1	1	2	0	7	1	0*	2
Skin: Wound	9	5	9	4	9	5	1*	3
All (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Bone marrow (femur): Increased haematopoiesis	9	3	10	10	9	2*	2*	2*
Bone marrow (sternum): Increased haematopoiesis	9	3	9	10	9	3	2*	2*
Bone marrow (Vertebra): Increased haematopoiesis	9	3	10	10	9	3	2*	2*
Lymph nodes (cervical): Plasma cell hyperplasia	6	2	8	5	8	3	1*	0*
Lymph nodes (mesenteric): Myeloid cell aggregation	5	0*	3	2	1	1	2	1
Spleen: Increased extramedullary haematopoiesis	20	7*	14	14	13	10	5*	9
Amyloid deposition	3	3	4	0	10	3*	0**	1**
Lung: Alveolar epithelial cell hyperplasia	0	5*	1	1	3	4	5	5
Small intestine: Amyloid deposition	2	1	1	0	8	0**	0**	3
Liver: Micro-granuloma	1	6	5	5	16	16	14	7*
Amyloid deposition	5	3	4	0*	12	3*	0**	1**
Kidney: Cortical cyst(s)	10	8	13	2*	5	1	5	0*
Glomerular amyloidosis	1	1	2	0	7	2	0**	2
Uterus: Amyloid deposition	-	-	-	-	6	0*	0*	1
Thyroid: Amyloid deposition	3	2	4	0	11	1**	0**	2**
Parathyroid[c]: Amyloid deposition	2	1	4	0	10	1**	0**	2*
Eye: Cataract	4	5	5	5	5	2	0*	2
Skin: Skin subcutaneous abscess	3	1	2	5	5	1	0*	1

Tk: Terminal kill; Ke/fd: Killed *in extremis* or found dead; All: All animals examined
(N=): Number of animals examined
* $p < 0.05$;

Table B.6.5.13-8: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in non-neoplastic lesions

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
78tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)

** p<0.01 (Fisher's exact probability test)
c: The number animals examined in the control, 1600, 8000 or 40000 ppm groups were 46, 48, 48 or 46 in males and 48, 48, 50 or 49 in females, respectively.

Neoplastic lesions
Table B.6.5.13-9 shows neoplastic lesions in the treated groups of either sex with statistically significant differences in incidence from those of the controls.

Table B.6.5.13-9: HR-001: 18-Month Oral Oncogenicity Study in Mice ([redacted] 1997): Statistically significant changes in histopathology findings: Incidence of malignant lymphoma at terminal sacrifice and over all animals

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
Hematopoietic & Lymphatic system: General: Malignant lymphoma								
All animals (N=)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
All animals: Incidence	2	2	0	6	6	4	8	7
All animals: Incidence [%]	4	4	0	12	12	8	16	14
Tk (N=)	(26)	(34)	(27)	(29)	(32)	(36)	(40)	(35)
Tk: Incidence	0	0	0	2	4	0*	5	3
Tk: Incidence [%]	0	0	0	7	12.5	0	12.5	8.5
HCD (7 studies between 1993 and 1998; 357/357 m/f mice examined in the 7 studies using the same diet MF Mash) [mean (range)]	7.0% (3.9-19.2%)				15.7% (7.8-26.9%)			

Tk Terminal kill; Ke/fd: Killed *in extremis* or found dead;
(N=) Number of animals examined;
* p < 0.05 (Fisher's exact probability test)

Historical control data for malignant lymphoma from the performing laboratory were submitted and presented in Table B.6.5.13-9 above. In male mice, the total incidence of malignant lymphoma in control groups varied considerably, ranging from 3.9% to 19.2%. In fact, 6 of 7 studies had a control incidence below 12% (6 % or lower) as observed now at the top dose level but, in principle, this incidence fell into the historical control range. The relevance of the apparent effect on malignant lymphoma with regard to the classification and labelling of glyphosate is discussed in Volume 1. In female control groups, malignant lymphoma incidence in the historical control data was between 8 and 27% (mean 15%) and, thus, the actual incidences in the control and treated groups were well covered.

In addition, two haemangiosarcomas and two renal tubule adenomas were observed in high dose males, in contrast to none in the mid, low and control group. This finding is further discussed in Volume 1.

Assessment and conclusion by applicant: Based on the effects in female mice on food consumption and body weight gain at the mid dose level of 8000 ppm, the lowest dose of 1600 ppm (ca. 153 mg/kg bw/day) is considered the NOAEL in this study. In contrast, the masses in lung mentioned in the dossier were not dose-related and there was no convincing evidence of lymph node swelling. Male mice appeared less vulnerable.

Assessment and conclusion by RMS:
Overall, the NOAEL is agreed with although it is noted that the study was conducted as a carcinogenicity study an only a limited number of parameters for systemic toxicity were evaluated.

B.6.5.14. Long-term toxicity – mouse, study 4

Data point:	CA 5.5/020 CA 5.5/021
Report author	[redacted]
Report year	1993 Note AGG : Applicant claimed 1989 as the report year. However, this was the start of the study and not the finalisation date of the study report. In Appendix E 1989 is used.
Report title	Glyphosate: 104-week dietary carcinogenicity study in mice
Report No	7793
Document No	154-GLY
Guidelines followed in study	US-EPA Pesticide Assessment Guidelines Subdivision F, 83-2 (1982); in general compliance with OECD 451
Deviations from current test guideline (OECD 451, 2018)	The following deviations were noted from the current OECD test guideline: - histopathological examinations were performed without Harderian gland, cervix, eyes, coagulating glands, submandibular lymph node, lacrimal glands, seminal vesicles and vagina.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant: Valid, Category 2a Conclusion AGG : Some minor deviations were noted compared to the current OECD test guideline, but overall the study is concluded to be acceptable for the endpoints evaluated.

The carcinogenic potential of glyphosate technical was assessed in a 104-week feeding study in male and female CD-1 mice. Groups of 50 mice per sex received daily dietary doses of 0, 100, 300 or 1000 mg/kg bw/day glyphosate technical for 24 months. Observations covered clinical signs, body weight, food and water consumption, differential blood count, as well as organ weights, necropsy and histopathological examination.

Achieved doses throughout the study period were generally close to nominal. There were no treatment-related deaths or clinical signs in any of the dose-groups. Body weight, food and water consumption did not differ significantly from the controls. Moreover, there were no treatment-related changes in differential blood count.

At necropsy the incidence of lung masses was slightly higher in the 1000 mg/kg bw/day group but no treatment related effect on histopathological findings were observed. Organ weight data showed marginal increased thymus weights in males at 300 and 1000 mg/kg bw/day after 104 weeks, but not in females and without corresponding histopathological changes. Histopathological examination noted increased mineral deposit in the brain of high dose males. These changes were not considered be toxicologically relevant. No treatment-related neoplastic lesions were observed at termination.

The NOAEL was concluded to be 1000 mg/kg bw/day, based on the absence of any adverse findings.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	206-JaK-25-1
Purity:	98.6%
Stability of test compound:	At least two years at ambient temperature in the dark

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	CD-1
Source:	[redacted]
Age:	Approx. 4 weeks upon arrival at testing facility
Sex:	Males and females
Weight at dosing:	Males: 30.9 ± 0.5 g, females: 23.5 ± 0.3 g
Acclimation period:	21 days
Diet/Food:	SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Either one male or one female in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 – 20 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-11-30 to 1991-12-23

Animal assignment and treatment:
In a carcinogenicity study groups of 50 CD-1 mice per sex received daily dietary doses of 0, 100, 300 or 1000 mg/kg bw/day glyphosate technical for 104 weeks. The dose levels were selected based on the results of a 13-week dietary toxicity study in mice.

Test diets were prepared once per week for the first 13 weeks and at least once every two weeks thereafter by direct admixture of the test substance to the plain diet and mixing for 20 minutes.

Analyses for achieved concentrations of the test substance in the diet were conducted from formulated diets or Weeks 1, 4, 8, 12, 15, 23, 30, 38, 46, 54, 62, 76, 83 and 93 of dosing. The stability and homogeneity of the test substance in the diet was determined prior to the start of the study.

Clinical observations
A check for mortality was made twice daily on all animals throughout the study. In addition, all animals were examined for clinical signs during each day. A detailed clinical examination and check for palpable masses were done once each week on every animal.

Body weight
Individual body weights were recorded for each animal before dosing, at weekly intervals until the end of week 13 and approximately every 4 weeks thereafter until termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 13 and subsequently every 4 weeks until termination. Water consumption was monitored by visual inspection throughout the study period.
Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology
During Weeks 52, 77 and 102 of dosing, a blood sample was taken from all surviving animals *via* tail snip without anaesthesia. Differential blood smears were prepared, fixed and stained from all animals. A differential blood count was performed on smears from all surviving Control and High dose animals at each time point.

The following parameters were measured: Differential leukocyte count (neutrophils, lymphocytes, monocytes and eosinophils). Absolute indices were calculated.

Sacrifice and pathology
All surviving animals were sacrificed and necropsied. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissections and necropsies were performed under the supervision of a pathologist, with the organs/tissues listed below being weighed and/or fixed.

The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostrate, salivary glands (parotid, sublingual and submaxillary), spleen, testes including epididymides, thymus and uterus.

The following organs were examined histopathologically: adrenals, aortic arch, any abnormal tissue, bladder, bone and bone marrow (sternum and rib), brain, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), heart, kidneys, liver with gall bladder, lungs, mammary gland, lymph nodes (mesenteric), muscle (thigh), oesophagus, ovaries, pancreas, salivary glands (parotid, submaxillary, sublingual), pituitary, prostrate, sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach (glandular and non-glandular), testes with epididymis, thymus, thyroid/parathyroid, trachea and uterus.

All tissues fixed with the exception of nasal cavity, seminal vesicles, submandibular lymph node, eyes, optic nerves, tongue, vagina, rib, blood smears and ears were processed and examined histologically from all animals in the Control and High dose groups and all premature decedents. Kidneys, liver, lungs and any abnormal tissue were examined from all other animals in the low and Intermediate dose groups.

Statistics
Body weight and organ weight data were statistically analysed for homogeneity of variance using the 'F-max' test. If the group variances appeared homogeneous, a parametric ANOVA was used and pairwise comparison made *via* Students's t-test using Fishers's F protected LSD. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous, then a nonparametric test such as Kruskal-Wallis ANOVA was used.

Organ weights were also analysed conditional on body weight (i.e. analysis of covariance). Differences in survival data between Control and groups receiving Glyphosate were assessed graphically using Kaplan-Meier plots. Histology and tumour data were analysed using Fisher's Exact Probability test.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Periodic analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10 %), with the exception of 2 instances where deviation of the mean from the theoretical concentration exceeded ± 10 % (Group 2♀ - Week 62: +248 % which was considered to have occured as a result of a sampling error and Group 3♀ - Week 93: -10.1 %).

B. MORTALITY
There were 208 pre-terminal deaths throughout the study. There was no evidence to suggest that any of these deaths were treatment-related.

The numbers of deaths are summarised in the table below.

Table B.6.5.14-1: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] *et al.*, 1989): Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Sex	Dose group (mg/kg bw/day)*			
	0	100	300	1000
Male	24/50	25/50	21/50	25/50
Female	29/50	34/50	24/50	26/50

* Number of dead / total number

C. CLINICAL OBSERVATIONS

There were no notable intergroup differences in either sex. The clinical signs seen were distributed equally throughout all groups and included emaciation, a hunched posture, subdued behaviour and exophthalmic eyes. These are considered to be typical for mice of this age and strain in a study of this type and duration.

There were no notable intergroup differences in the incidences of externally palpable masses.

D. BODY WEIGHT

Generally, slight (incidental) increases of body weights were observed in substance treated animals of all dose groups until week 52. The effect was most often observed in high dose males being constantly about 10% and less often observed in high dose females. Between week 52 and 104 the body weights were comparable within all groups. All groups receiving glyphosate showed a comparable weight gain to that of their respective controls. The mean body weight gain data are summarised in the table below.

Table B.6.5.14-2: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] *et al.*, 1989): Body weight development (mean values) after 104-week dietary exposure to glyphosate technical

	Dose group (mg/kg bw/day)							
	0		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain (g) 0-104 weeks	12.8	14.9	↑13.1	↓14.1	↑14.4	↓14.7	↑13.0	↑15.2
% of control	--	--	102	95	113	99	102	102

E. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food and water consumption for either sex noted during the study. The overall group mean achieved doses are summarised in table below.

Table B.6.5.14-3: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] *et al.*, 1989): Group mean achieved dose levels – oncogenicity study

Dose group	Nominal dose (mg/kg bw/day)	Mean achieved dose level (mg/kg bw/day)		Mean achieved dose level (% of nominal)	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	100	98 ± 6	102 ± 11	98	102
3 (mid)	300	297 ± 17	298 ± 30	99	99
5 (high)	1000	988 ± 56	1000 ± 113	99	100

Over the entire study duration the mean achieved dosages in all groups were close to the nominal.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology – Differential Blood Counts
There were no notable intergroup differences in either sex at any time point.

H. NECROPSY

Gross pathology
The incidence of lung masses was slightly higher in the male high dose group (18/50) compared to the male control (10/50). There were no other findings in males or females noted at necropsy that could be related to treatment with Glyphosate.

Organ weights
Absolute thymus weight was increased in the male intermediate and high dose groups ($p < 0.01$ and $p < 0.05$, respectively) compared to control. Thymus weight was also increased in the male intermediate and high dose groups ($p < 0.05$ and $p < 0.01$, respectively) after covariance analysis.

There were no other notable intergroup differences in males or females.

Table B.6.5.14-4: Glyphosate – 104 week dietary carcinogenicity study in mice ([redacted] *et al.*, 1989): Absolute thymus weights

Dose group	Nominal dose (mg/kg bw/day)	absolute Thymus weight (g)	
		males	females
control	0	0.02 ± 0.01	0.02 ± 0.01
2 (low)	100	0.02 ± 0.01	0.05 ± 0.05
3 (mid)	300	**0.03 ± 0.02****	0.04 ± 0.03
5 (high)	1000	**0.03 ± 0.03***	0.06 ± 0.08

* Significantly different from control, $p < 0.05$;
** Significantly different from control, $p < 0.01$

Histopathology

Non-neoplastic changes
The incidence of increased mineral deposits in the brain was higher in the male high dose group (13/50, $p < 0.05$) compared to control (4/49). In females, no significant increase was observed however did appear to be slightly higher (8/39 versus 4/40 in control).

A significant increase in kidney cysts was observed in mid dose males (22/50 versus 11/50 in control). However, since no dose response relationship occurred this effect was not considered to be treatment related.

Neoplastic findings
There were no statistically significant increases in incidence of any tumour.
The number of animals with tumours, both benign and malignant, was similar between the control and high dose groups of both sexes. 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 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 control (males: 49 and females: 36).

Hemangiosarcoma was evident in 4/50 high dose males, 2/50 low dose females and 1/50 high dose females (not significant) compared to the respective controls (both 0/50). The incidence was within the historical control incidence from the same lab (mean 3.3%, range 0-8%, 6 studies terminated between September 1988 and September 1991) and this finding was not observed in the other carcinogenicity studies. Therefore, it was considered to be incidental.

Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue was evident in 2/50 low and 2/50 high dose males and 3/50 low, 3/50 intermediate and 1/50 high dose females (not significant) compared to the respective controls (both sexes 0/50). Considering the lack of a dose-response relationship it was not considered to be treatment

related. Further, the incidence was within the historical control incidence from the same lab (mean 3.2%, range 0-8%, 6 studies terminated between September 1988 and September 1991).

Malignant lymphoma was reported at the following incidences in males: 4/50, 2/25, 1/21 and 6/50 for the control, low, mid and high dose, respectively. In females, the reported incidences of malignant lymphoma are 14/50, 12/34, 9/24 and 13/50, respectively. It should be noted that not all animals from low and mid dose levels were examined; only the animals that died during the study or that were killed in extremis were investigated in these groups, therefore no comparison can be made for these dose groups.

Other tumours seen were considered to be typical for mice of this age and strain due to the very low incidence of occurrence and were not considered to be due to administration of glyphosate.

Assessment and conclusion by applicant: In conclusion, glyphosate technical was not carcinogenic in male and female CD-1 mice following continuous dietary exposure of up to 1000 mg/kg bw/day (the limit dose for this type of study) for 104 weeks. Based on the study results and the lack of toxicological relevance of the thymus weight findings, as well as an increase of mineral deposit in the brain observed at 1000 mg/kg bw/day, the NOAEL in rats after chronic exposure to glyphosate technical for 104 weeks is considered to be 1000 mg/kg bw/day.

Assessment and conclusion by RMS:
The increase in thymus weight was only slight and without corresponding histopathological changes and therefore not considered to be toxicologically relevant. Mineral deposits are a common finding and are also considered to be of limited toxicological relevance. Overall, the NOAEL of 1000 mg/kg bw/day is agreed with. It is noted that only a limited number of general toxicity parameters were investigated as the study was set up as a carcinogenicity study and not a chronic toxicity study.

B.6.5.15. Long-term toxicity – mouse, study 5

Data point:	CA 5.5/022
Report author	[redacted]
Report year	1988
Report title	Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted]
Report No	Not reported
Document No	Not reported
Guidelines followed in study	No guideline followed; similar to OECD 453 (1981)
Deviations from current test guideline (OECD 453, 2018)	The following deviations were noted from the current OECD test guideline: - less than 50 (25) animals/sex/dose were used for the main test and less than 10 (5) animals/sex/dose were used as a satellite group; - Mortality checked daily at the start of the study instead of twice daily. - no ophthalmology performed; - haematology was performed without determining mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time; - clinical chemistry was performed without determining inorganic phosphorous, calcium, chloride, sodium, potassium, cholesterol, AST, any hepatobiliary evaluation, albumin, creatinine, - organ weights of brain, epididymides, spleen, (para)thyroids and uterus were not determined; - histopathology was performed without determining bone/bone marrow, caecum, harderian gland, cervix, coagulating gland, epididymides, jejunum, lacrimal gland, rectum, mammary gland, peripheral nerve, prostate, skeletal muscle, spinal cord, trachea, vagina. - In most cases histopathology was reported to be within normal limits instead of providing exact numbers.

	- Statistical analysis not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Not reported
Acceptability/Reliability:	Conclusion applicant: Invalid (category 3b) Due to restrictions regarding dose levels, number of animals used and insufficient investigations the study is not considered acceptable for hazard and risk assessment. In view of the discussion ongoing on the endpoint carcinogenicity a full summary is provided in order to allow a complete re-evaluation. The test substance (batch reference or purity) was also not clearly defined in the report. Conclusion AGG : A large number of parameters required under OECD 451 were not included. Moreover the number of animals were too low to allow a reliable conclusion on the toxicological properties of glyphosate. It is also noted that the dose level is quite low compared to the other mice studies. Furthermore the background tumour incidences in the study are unusually low. Overall, the study is concluded to be unacceptable.

Glyphosate (Technical) manufactured by [redacted] was administered *via* the diet in concentrations of 75, 150 or 300 ppm (equal to mean achieved dose levels of 0, 1.63, 3.35 or 5.87 mg/kg bw/day (males) and 0, 1.65, 3.35 or 5.42 mg/kg bw/day (females)) to 25 Balb/c mice per sex and dose for 80 weeks. In addition 5 animals/sex/dose were used as a satellite group and were treated for 40 month.

Animals were observed for any abnormal toxicity, body weight, food consumption, haematology, clinical chemistry, organ weights, histopathology and occurrence of tumours.

No toxic symptoms were noted during the study. The body weight and the food intake of the high dose animals of both sex was reduced. Haematology and clinical chemistry values of all treated groups were comparable to the control. No significant differences were found in organ weights or histopathological examination and no abnormal rise in tumour occurrence was found in the animals fed with the test material.
Glyphosate (Technical) supplied by [redacted], did not show any significant increase in tumour formation or toxicological effects when Balb/c mice were fed at the dose of 300 ppm (mixed with food) for 80 weeks.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	No data given in the report
Lot/Batch #:	No data given in the report
Purity:	No data given in the report
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Diet

3. Test animals:

Species:	Albino mouse
Strain:	Balb/c inbred
Source:	No data given in the report

Age:	5-8 weeks
Sex:	Males and females
Weight at dosing:	Males: 11.64 – 12.24 g, females: 11.30 – 12.04 g
Acclimation period:	No data given in the report
Diet/Food:	Pelleted food (Lipton India Ltd, India), mixed with the test material
Water:	Water, *ad libitum*
Housing:	Initially in groups of five in polypropylene cages
Environmental conditions:	Temperature: 19 - 25 °C Humidity: 30 - 70 % Air changes: No data given in the report 12 hours light/dark cycle

B: Study design and methods

In life dates: Not reported

Animal assignment and treatment:
In a combined chronic toxicity and carcinogenicity study groups of 25 Balb/c mice per sex received daily dietary doses of 0, 75, 150 and 300 ppm (equivalent to mean achieved dose levels of 0, 1.63, 3.35 and 5.87 mg/kg bw/day (males) and 0, 1.65, 3.35 and 5.42 mg/kg bw/day (females)) Glyphosate technical for 80 weeks. In addition, for the control and each dose group 5 rats per sex were included for interim sacrifice in month 9 to study blood chemistry and haematology (chronic toxicity study).

Clinical observations
General observations were performed once a day to start with and twice daily later on to prevent death of animals at night. Animals were palpated for occurrence and size of the tumour.

Body weight
Individual body weights were recorded on Day 0, once a week until the end of Week 13 and every 4 weeks thereafter until termination.

Food consumption and compound intake
Food consumption was recorded once weekly for each group from Week 1 to Week 13 and then every 4 weeks.

Haematology and clinical chemistry
The satellite group of 10 animals was sacrificed at the end of 9 months, blood was collected and processed for the following haematological and biochemical studies. All the surviving animals were sacrificed at the end of 18 months, their blood was collected and processed for haematological and biochemical studies.

Haematology
The following parameters were measured: Haemoglobin, total red blood cell count, packed cell volume (PCV), platelets, total white blood cell count and differential white blood cell count.

Blood chemistry
The following parameters were measured: Total serum proteins, alanine transaminase (ALT), blood urea nitrogen and blood glucose.

Sacrifice and pathology
Necropsy was performed on all animals that died during the observation period and all animals at scheduled termination.

The following absolute and relative (percent of body weight) organ weights were determined: adrenals, heart, gonads, kidneys, liver and spleen.

Tissue samples for histopathology were taken from the following organs of all animals: adrenals, aorta, brain, colon, duodenum, eyes, gall bladder, heart, ileum, kidneys, liver, lungs, lymph nodes, oesophagus, ovaries, pancreas, pituitary, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus, thyroid, urinary bladder, uterus and gross pathological lesions.

Statistics
Not reported.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analysis of the dose formulation was not performed.

B. MORTALITY
No treatment-related deaths were observed in this study.

The numbers of pre-terminal deaths in the main group are displayed in the below **Table** :

Table B.6.5.15-1: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted] ([redacted] 1988): Cumulated mortalities after 80-week dietary exposure to Glyphosate technical

Sex	Dose group (ppm)			
	0	75	150	300
Male	4/25	7/25	5/25	7/25
Female	4/25	5/25	3/25	7/25

C. CLINICAL OBSERVATIONS
None of the animals under treatment exhibited any toxic symptoms during the course of the study.

D. BODY WEIGHT
The gain in body weight of animals treated with Glyphosate (Technical) at two dose levels 75 ppm and 150 ppm was found to be comparable with that of control animals, while that of animals in the higher dose group (i.e. fed with 300 ppm) was found to be slightly reduced (<10%).

Table B.6.5.15-2: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted] ([redacted] 1988): Group mean body weights

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	75	150	300	0	75	150	300
	No. of rats	25	25	25	25	25	25	25	25
Week 0		12.00 ± 0.32	↑12.24 ± 0.30	↓11.72 ± 0.27	↓11.64 ± 0.23	11.80 ± 0.29	↑12.04 ± 0.29	↓11.30 ± 0.21	↑11.90 ± 0.30
Week 1		13.88 ± 0.33	↑14.16 ± 0.36	↓13.12 ± 0.35	↓13.28 ± 0.24	14.00 ± 0.37	↑14.10 ± 0.35	↓13.10 ± 0.32	↓13.20 ± 0.39
Week 2		15.48 ± 0.28	↑15.80 ± 0.37	↓13.24 ± 0.28	↓15.00 ± 0.28	16.00 ± 0.33	↑16.20 ± 0.31	↓15.00 ± 0.22	↓15.00 ± 0.40
Week 3		16.80 ± 0.25	↑17.37 ± 0.39	↓16.60 ± 0.31	↓16.52 ± 0.28	17.70 ± 0.32	↑18.30 ± 0.30	↓16.10 ± 0.26	↓16.60 ± 0.41
Week 13		26.24 ± 0.32	↑26.48 ± 0.27	↓25.72 ± 0.45	↓25.72 ± 0.42	27.00 ± 0.31	↑27.60 ± 0.60	↓25.60 ± 0.23	↓25.60 ± 0.54
Week 53		34.79 ± 0.72	↑34.96 ± 0.71	↓34.10 ± 0.48	↓33.10 ± 0.34	33.69 ± 0.67	↑35.12 ± 0.70	↑34.46 ± 0.47	↓33.40 ± 0.52

Table B.6.5.15-2: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted] ([redacted] 1988): Group mean body weights

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	75	150	300	0	75	150	300
	No. of rats	25	25	25	25	25	25	25	25
Week 80		41.70 ± 1.20	↑42.44 ± 1.18	↓40.60 ± 0.79	↓37.83 ± 0.70	39.62 ± 1.01	↑45.25 ± 1.04	↑41.36 ± 0.73	↓36.50 ± 0.56

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Glyphosate (Technical) up to the dose of 150 ppm did not cause any effect on food consumption while the animals in the high group (300 ppm) showed reduction in food intake (see table below). The mean intake for each dose group is 1.63, 3.35 and 5.87 mg/kg bw/day (males) and 1.65, 3.35 and 5.42 mg/kg bw/day (females) for 75, 150 and 300 ppm, respectively.

The group mean achieved doses are summarised below.

Table B.6.5.15-3: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted] ([redacted] 1988): Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)	
		Males	Females
low	75	1.63	1.65
mid	150	3.35	3.35
high	300	5.87	5.42

Table B.6.5.15-4: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted] ([redacted] 1988): Group mean food consumption

Timepoint	Sex	Males				Females			
	Dose (ppm)	0	75	150	300	0	75	150	300
	No. of rats	25	25	25	25	25	25	25	25
Week 1		2.20	↓2.16	↓2.16	2.20	2.16	↑2.24	2.16	↑2.28
Week 80		4.71	↑5.00	↓4.30	↓3.00	4.38	↑4.95	↓3.77	↓2.72

F. LABORATORY INVESTIGATION

Haematology

The values of haematological parameters in the animals of treated groups (Groups II, III and IV) were found to be comparable to the animals in the control group (Group I) at interim and final sacrifice.

Clinical chemistry

The levels of ALT, blood urea nitrogen, total serum proteins and blood glucose were found to be comparable in all the animals at interim and final sacrifice.

G. NECROPSY

Organ weights

Absolute and relative values of organ weights of treated animals of (groups II, III and IV) were not significantly different from those of control animals of Group I. It is noted that the absolute organ weight of the testes could not be read for group II due to the low quality of the study report.

Histopathology
Histopathological changes were found at all dose levels including control without showing dose response, hence it is concluded that these are no treatment-related effects. No summary table is provided in the study report. The individual animals data was checked by the AGG and indeed no dose-related effect was observed but it appears some animals are missing in the results, e.g. in control females only results for 11 animals were reported.

Neoplastic changes
The incidence of tumour finding was comparable in control animals and animals treated at various dose levels. The summary text of the study report list the incidence in the low and mid dose males and control and mid dose females to be out of 24/25 animals. However, individual animal data could not be found for a number of animals in the study report. Therefore, the incidences in the table below is based on the individual animal data only. It is noted by the RMS that the reported background tumours incidence appears to be unusually low.

Table B.6.5.15-4: Carcinogenicity and Chronic Toxicity Study of Glyphosate (Technical) of [redacted] ([redacted] 1988): Neoplastic changes

ppm	Males				Females			
	0	**75**	**150**	**300**	**0**	**75**	**150**	**300**
Liver: Hepatocellular adenoma	1/25	1/5	0/15	1/24	1/11	0/25	1/14	1/25
Lung: Alveolar adenoma	1/25	1/5	1/15	2/24	1/11	1/25	0/14	1/25

Assessment and conclusion by applicant:
Balb/c mice did not show any significant increase in tumour formation or toxicological effects when given Glyphosate (technical) in the diet at up to 300 ppm (equivalent to 5.87 or 5.42 mg/kg/day in males and females respectively for 80 weeks.
Due to several limitations the study is not considered acceptable for hazard and risk assessment.

Assessment and conclusion by RMS:
Due to the severe limitations no conclusion can be drawn on the basis of this study. This conclusion is in line with the previous EU evaluation.

B.6.5.16. Long-term toxicity – mouse, study 6

Data point:	CA 5.5/023
Report author	[redacted].
Report year	1983
Report title	A chronic feeding study of glyphosate (Roundup® technical) in mice
Report No	77-2061
Document No	M-646425-01-1
Guidelines followed in study	No guideline specified; in general compliance with OECD 451
Deviations from current test guideline (OECD 451, 2018)	The following deviations were noted : - Histopathological examinations were performed without coagulating glands, lacrimal glands, seminal vesicles and vagina.
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP; no certificate)

Acceptability/Reliability:	Conclusion applicant: Valid (category 2a) Conclusion AGG: The minor deviations compared to the current test guideline are not considered to be critical and therefore the study is concluded to be acceptable

This study was designed to assess the oncogenic potential and toxicity of glyphosate (Roundup® Technical) when administered orally, *via* dietary admixture to CD-1 mice (50/sex/group) at dose levels of 1000, 5000 or 30000 ppm (equivalent to 157, 814 and 4841 mg/kg bw/day for males and 190, 955 and 5874 mg/kg bw/day for females) for a period of twenty-four months. Control animals received basal diet.

Mean body weights for the high-dose males were generally lower than control; differences from control were as great as -11 % (at Week 102) and were, for the most part, statistically significant. Mean body weights for the high-dose females and the males and females at the low- and mid-dose levels did not demonstrate a response to treatment.

Other parameters evaluated, i.e. general animal condition, body weight gain. food consumption, feed efficiency, water consumption and haematology revealed no consistent dose- or treatment-related response to administration of glyphosate.
At the terminal sacrifice, the mean absolute and relative (to body and brain weights) weight of the testes were elevated for the high-dose group. Other organ weight differences noted were attributed to differences in body weight or were sporadic and were not considered treatment-related.

Correlation of necropsy observations with microscopic findings revealed no treatment relationship.

Neoplastic findings were those commonly encountered in mice. Bronchioalveolar tumours of the lungs, hepatocellular neoplasms, and tumours of the lymphoreticular system accounted for the majority of those encountered. There were no suspected test substance-associated trends in the incidence of these tumours or in any of the other spontaneously occurring neoplasms.

The other neoplasms that occurred with any frequency in treated mice only were renal tubule adenomas, which occurred in males. Three were present at the high-dose and one at the mid-dose level;, the distribution of this benign tumour was considered spurious and unrelated to treatment in the study report. However, it is noted that no historical control data is available. All other neoplasms occurred sporadically and were considered to have had no relationship to treatment.

Of the non-neoplastic findings, hepatic central lobular hypertrophy and necrosis was noted with increased incidence in the high-dose males. In addition a significant increase in chronic interstitial nephritis was noted in high dose males. In females an increase of proximal tubule epithelial basophilia and hypertrophy were observed in high dose females. In males, at the mid and high dose slight to mild epithelial hyperplasia in the urinary bladder was observed.

All other tissue alterations occurred sporadically or were considered to have been spurious in distribution. Most occurred with approximately equal frequency and severity in control and treated mice and were judged to be unrelated to glyphosate administration.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (Roundup® technical)
Description:	Fine, white clumped powder
Lot/Batch #:	NB 1782608/3 and NB 1782610/7
Purity:	99.7 %

Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Diet
3. Test animals:	
Species:	Mouse
Strain:	CD-1, COBS (ICR derived)
Source:	[redacted]
Age:	at receipt: 29 days; at treatment: 40 days
Sex:	Males and females
Weight at dosing:	Males: 23 g (16 – 28 g); females: 20 g (15 – 24 g)
Acclimation period:	11 days
Diet/Food:	Purina® Rodent Laboratory Chow #5001, *ad libitum*
Water:	automated watering system (Elizabethtown Water Company), *ad libitum*
Housing:	Animals were doubly housed in elevated stainless steel wire mesh cages during the first week of the acclimation period and individually housed thereafter.
Environmental conditions:	Temperature: 18.3 – 23.9 °C Humidity: 30 – 80 % Air changes: not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1980-03-31 to 1982-03-14

Animal assignment and treatment:
In a carcinogenicity study groups of 50 CD-1 mice per sex received daily dietary doses of 0, 1000, 5000 or 30000 ppm (equal to 157, 814 and 4841 mg/kg bw/day for males and 190, 955 and 5874 mg/kg bw/day for females) glyphosate technical for 104 weeks.

Test diets were prepared once per week. Analyses for achieved concentrations of the test substance in the diet were conducted by the sponsor.

Clinical observations
A check for mortality and for gross signs of toxicology was made twice daily on all animals throughout the study. A detailed clinical examination and check were performed at the beginning of the study and once each week on every animal.

Body weight
Individual body weights were recorded for each animal twice before dosing, at weekly intervals until the end of week 14, biweekly thereafter until termination and at termination.

Food and water consumption and compound intake
Food consumption was recorded once weekly for each cage group starting one week before treatment until Week 14 and subsequently biweekly until termination.

Water consumption measured and recorded daily for 10 animals/sex/group at month 12 over one 3-day interval. A second 3-day interval, at month 12, was omitted due to technician error. Due to the high mortality across all groups during month 24, water consumption measurements were initiated on 12 animals/sex/group in an attempt to insure adequate sample size for statistical evaluation. Measurements at month 24 were performed over one 3-day and one 2-day interval.

Achieved dosages were calculated from nominal dietary concentration, taking into account actual food consumption and body weight data.

Haematology
During Month 12, 18 and 24 of dosing, blood was obtained *via* venipuncture of the orbital sinus (retrobulbar venous plexus) under light ether anaesthesia. Animals were selected randomly; the same animals were used at all intervals when feasible. Mice were fasted overnight prior to blood collections and were not fed until after samples were collected.

Performed on 10 animals/sex/group, evaluations were conducted on 12 males/group (to insure adequate sample size for statistical evaluation) and on all females surviving to the last day of sacrifice (mortality occurring during the water consumption measurement period resulted in haematology evaluations on fewer than 10 animals/group in some groups).

The following parameters were measured: Haemoglobin, haematocrit, erythrocytes, platelets, total and differential leukocytes, erythrocyte morphology.

Sacrifice and pathology
Complete gross post-mortem examination was performed on all animals which died spontaneously, accidentally, or were killed moribund as well as at termination. External surface, all orifices, the cranial cavity, carcass, the external surfaces of the brain and spinal cord, nasal cavity and paranasal sinuses, the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs were examined for all animals. Animals were fasted prior to the terminal sacrifice.

The following organs were weighed: adrenals, brain (with entire brain stem), heart, kidneys, liver, ovaries, spleen and testes without epididymides.

The following organs were examined histopathologically: adrenals, abdominal aorta, bladder, bone and bone marrow (costochondral junction), bone marrow smear and blood smear, brain, epididymides, eyes (with optic nerve and contiguous Harderian glands), gross lesions, gall bladder, head, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidneys, liver, lungs, mammary gland, lymph nodes (mediastinal and mesenteric), skeletal muscle (biceps femoris), oesophagus, ovaries, pancreas, mandibular salivary glands, pituitary, prostrate, sciatic nerve, skin, spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tissue masses or suspect tumours and regional lymph nodes, trachea and uterus (with cervix).

Statistics
Body weight, body weight gain, food and water consumption, feed efficiency, haematology parameters, terminal organ and body weights, organ/body and organ/brain weight ratios were analysed. Mean values of all dose groups were compared to control at each time interval. Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances appeared homogeneous a parametric ANOVA was used using the F distribution to assign significance. If significant difference among the means were indicated Dunnett's test was used to determine which means were significantly different from the controls. If a nonparametric procedure for testing equality of mean was needed, the Kruskal-Wallis test was used. A statistical test for trend in the dose levels (Cochran) was also performed.

II. RESULTS

A. ANALYSIS OF DOSE FORMULATIONS
Analyses for achieved concentrations of the test substance in the diet were conducted by the sponsor.

B. MORTALITY
The incidence of mortality was low during the first 18 months of study, with survival rates greater than or equal to 62 % and 68 % for the males and females, respectively. The mortality rate increased after 18 months, as would be expected in aging mice. The incidence of mortality was considered to demonstrate no dose- or test substance-related adverse effect of glyphosate administration on survival, i.e. survival was greater than control in the high-dose males and mid- and high-dose females, with the lowest survival in the low-dose females.

The numbers of deaths are summarised in the table below.

Table B.6.5.16-1: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Cumulated mortalities after 104-week dietary exposure to glyphosate technical

Sex	Dose group (ppm)[1]			
	0	**1000**	**5000**	**30000**
Male	27/50 (3)	32/50 (2)	32/50 (1)	23/50 (1)
Female	27/50 (3)	38/50 (0)	22/50 (1)	23/50 (4)

[1] Number of dead / total number; accidental deaths are presented in parentheses

C. CLINICAL OBSERVATIONS

The physical observations noted throughout the study were considered common for CD-l mice under laboratory conditions; these observations included yellow staining of the anogenital area, dermatological abnormalities (scabbing on the ears and alopecia), excessive lacrimation, displacement of the pupils and ocular opacities. These observations occurred with low incidence and did not occur in a pattern suggestive of a relationship to treatment.

D. BODY WEIGHT

In high dose males a fairly consistent decrease in body weight was observed, with a terminal body weight that is 11% lower than controls.

Sporadic differences from control were noted in the mean body weight data for the high-dose females, and the males and females at the low- and mid-dose levels. However, these differences from control were slight and failed to demonstrate a consistent pattern with respect to treatment level over time, and were therefore, not considered to reflect a response to treatment.

Table B.6.5.16-2: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Mean body weights

ppm		Males				Females			
		0	**1000**	**5000**	**30000**	**0**	**1000**	**5000**	**30000**
Week -1	Mean body weight (g)	21.5	21.5	↓21.4	↑21.6	19.4	↓19.3	19.4	↓19.3
	Std. Dev.	1.2	1.3	1.2	1.5	1.7	1.6	1.5	1.8
Week 0	Mean body weight (g)	22.6	↑22.8	↓22.5	↓22.5	20.3	↑20.5	↓19.9	↓19.6
	Std. Dev.	1.3	1.4	1.8	1.7	1.4	1.5	1.7	1.4
Week 1	Mean body weight (g)	25.8	↓25.5	↓25.0	↓**24.5****	22.0	↑22.6	↑22.4	↑22.4
	Std. Dev.	1.4	1.6	2.0	1.8	1.5	1.5	1.7	1.4
Week 2	Mean body weight (g)	29.1	↓**27.7****	↓**27.8****	↓**26.1****	22.5	↑**23.4***	↑23.2	↑**23.9****
	Std. Dev.	1.8	1.8	2.2	1.9	1.5	1.5	1.7	1.6
Week 3	Mean body weight (g)	28.7	↑28.8	↑29.2	↓**27.5***	23.7	↑**24.5***	↑24.4	↑**24.8****
	Std. Dev.	1.6	1.9	2.3	2.1	1.8	1.5	1.8	1.8
Week 13	Mean body weight (g)	34.8	↓**33.3***	↑35.1	↓**33.2****	28.9	↑29.5	↑29.3	↓28.8
	Std. Dev.	2.4	2.6	2.8	2.5	1.8	2.1	2.1	2.0
Week 24	Mean body weight (g)	35.6	↓34.7	↓35.3	↓35.5	31.0	↑31.3	↓30.7	↓30.9
	Std. Dev.	2.3	3.3	3.2	2.5	2.2	2.6	2.5	2.4
Week 52	Mean body weight (g)	36.4	↓35.0	↓36.1	↓**33.8****	32.3	↑33.1	↓**30.0****	↓32.1
	Std. Dev.	2.7	2.8	3.1	2.8	2.8	2.9	3.2	2.5

Table B.6.5.16-2: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Mean body weights

ppm		Males				Females			
		0	**1000**	**5000**	**30000**	**0**	**1000**	**5000**	**30000**
Week 78	Mean body weight (g)	38.0	↓37.6	↓37.4	↓**35.6***	33.1	↑**35.5**§	↑33.4	↓31.5
	Std. Dev.	3.3	3.7	4.7	3.0	3.0	3.9	4.2	2.5
Week 100	Mean body weight (g)	36.4	↑**39.6***	↑**39.7****	↑36.6	35.1	↑37.6	↑35.7	↓33.6
	Std. Dev.	2.4	2.9	1.9	2.7	3.2	5.6	4.9	2.7
Week 102	Mean body weight (g)	37.7	↑37.9	↓35.7	↓**33.6****	-	-	-	-
	Std. Dev.	2.6	3.6	2.5	3.6	-	-	-	-

* Significantly different from control (Dunnett's test; $p \leq 0.05$)
** Significantly different from control (Dunnett's test; $p \leq 0.01$)
§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.05$)
§§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

E. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food and water consumption for either sex noted during the study. Test substance intake was calculated from individual body weight and food consumption values and nominal concentrations of 1000, 5000 and 30000 ppm of glyphosate and expressed as milligrams of test substance per kilogram of body weight per day (mg/kg/day).

The overall group mean achieved doses are summarised in the table below.

Table B.6.5.16-3: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Summary of food consumption data

ppm		Males				Females			
		0	**1000**	**5000**	**30000**	**0**	**1000**	**5000**	**30000**
Week 0	Mean (g)	272.8	↑280.9	↑274.5	↓265.4	273.2	↓269.6	↓270.0	↑297.9
	Std. Dev.	36.6	40.4	42.6	54.0	60.5	64.6	50.3	80.1
Week 1	Mean (g)	250.7	↓249.9	↑252.8	↓**240.7**§§	287.3	↑287.8	↓**249.1**§§	↑328.6
	Std. Dev.	17.0	27.8	29.6	46.9	69.5	53.8	53.6	101.4
Week 2	Mean (g)	211.2	↑**230.6****	↑**232.8****	↑**234.7****	266.0	↑284.3	↓264.3	↑**298.7**§§
	Std. Dev.	20.4	16.8	19.0	21.4	32.0	52.0	30.9	53.2
Week 3	Mean (g)	202.0	↓197.7	↑208.7	↓197.1	246.4	↓244.2	↑260.8	↑289.8
	Std. Dev.	22.1	22.3	27.3	29.1	29.9	54.3	36.4	84.4
Week 13	Mean (g)	149.9	↑**163.7****	↑150.2	↑**161.1****	186.5	↑190.8	↑198.5	↑**215.6****
	Std. Dev.	12.3	17.7	12.2	12.7	27.4	46.8	19.0	48.6
Week 24	Mean (g)	162.4	↑164.8	↑**183.3**§§	↑170.2	171.1	↑181.9	↑**186.9***	↑**194.5****
	Std. Dev.	10.7	17.0	23.6	19.5	23.4	26.3	23.4	32.2
Week 52	Mean (g)	176.2	↓172.7	↓**164.3****	↓171.8	187.2	↑194.6	↑**226.5**§§	↓**149.5**§§
	Std. Dev.	12.8	15.3	16.3	13.9	18.9	42.3	24.4	29.9
Week 78	Mean (g)	146.5	↓137.3	↓143.9	↑**161.3****	165.7	↓153.7	↑172.7	↑173.2
	Std. Dev.	20.3	19.5	25.4	18.7	32.1	26.2	20.8	26.6
Week 98	Mean (g)	145.2	↑153.8	↑159.0	↑**178.5**§§	165.1	↑**187.6****	↑165.5	↑177.6
	Std. Dev.	20.6	46.9	46.8	62.1	28.9	25.3	20.7	22.1
Week 100	Mean (g)	158.5	↓**119.1****	↓**103.9****	↓**115.5****	164.7	↓**143.8****	↓**144.4****	↓**141.1****
	Std. Dev.	11.1	11.5	11.8	14.8	18.3	21.6	16.1	17.6
Week 102	Mean (g)	139.2	↑140.7	↑145.2	↑145.0	-	-	-	-
	Std. Dev.	20.1	20.5	27.6	17.4	-	-	-	-

* Significantly different from control (Dunnett's test; $p \leq 0.05$)
** Significantly different from control (Dunnett's test; $p \leq 0.01$)
§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.05$)
§§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

Table B.6.5.16-4: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Group mean achieved dose levels – oncogenicity study

Dose group	Concentration (ppm)	Mean achieved dose level ranges (mg/kg bw/day)		Mean achieved dose level according to study report (mg/kg bw/day)	
		Males	Females	Males	Females
1 (control)	0	--	--	--	--
2 (low)	1000	110.9 – 249.9	128.9 – 287.8	157	190
3 (mid)	5000	519.3 – 1264.2	689.7 – 1321.5	814	955
4 (high)	30000	3465.0 – 7219.8	4232.4 – 9858.6	4841	5874

Mean water consumption values (expressed in millilitres of water consumed per kilogram of body weight per day) for the treated males at month 12 were lower than control values however, without showing a dose-response (242, 217, 170 and 210 ml/kg bw/day at 0, 1000, 5000 and 30000 ppm). , In addition, differences from control were not statistically significant and at month 24, values for the treated males (particularly the high-dose group) were comparable to control values (140 vs 141 in control). Water consumption values for the low- and mid-dose females were somewhat elevated at month 12 relative to control, without dose response (272, 319, 379 and 297 ml/kg bw/day) and a similar pattern was not evident at month 24. No consistent test substance-related effects on water consumption were apparent in these data.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Although a few statistically significant differences from control were noted for some of the haematology parameters evaluated, these differences occurred sporadically or did not occur in a dose-related pattern and were not consistent over time. Therefore, these slight differences were not considered to be of toxicological significance.

Statistically significant findings are included in the table below.

Table B.6.5.16-5: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983):: Summary of haematological data

ppm		Males				Females			
		0	1000	5000	30000	0	1000	5000	30000
Plat									
Month 12	Mean (10^5/mm³)	16.05	↑**18.17***	↓14.75	↑17.48	14.00	↑15.84	↑16.17	↓13.43
	Std. Dev.	1.75	0.97	1.51	1.69	2.02	3.32	5.04	3.41
Month 18	Mean (10^5/mm³)	16.42	↑19.86	↓13.86	↑16.70	11.12	↑12.15	↑11.32	↑11.17
	Std. Dev.	5.13	5.37	3.21	3.18	2.15	1.89	3.09	2.05
Month 24	Mean (10^5/mm³)	16.97	↓16.90	↓14.41	↓15.42	9.27	↑12.42	↑10.51	↓8.58
	Std. Dev.	4.85	5.78	4.44	6.16	2.27	5.04	3.68	3.21
RBC									
Month 12	Mean (10^3/mm³)	5.5	↑6.1	↑5.9	↓**3.3***	4.3	↑4.6	↑4.7	↓3.9
	Std. Dev.	1.8	2.6	2.0	1.4	2.8	2.8	2.8	2.1
Month 18	Mean (10^3/mm³)	4.2	↑5.6	↑4.4	4.2	5.9	↓4.7	↓4.8	↓4.4
	Std. Dev.	1.5	2.6	2.8	2.1	2.3	1.8	2.9	2.0
Month 24	Mean (10^3/mm³)	4.5	↑5.5	↓3.9	↓3.9	4.2	↓4.1	↓3.9	↓3.4
	Std. Dev.	1.8	2.6	1.6	1.9	1.1	2.0	2.9	1.0

* Significantly different from control (Dunnett's test; $p \leq 0.05$)

H. NECROPSY

Gross pathology
Correlation of necropsy observations with microscopic findings revealed no trend indicating a test substance relationship.

Organ weights
Differences in mean absolute or relative (to body or brain weights) organ weights from control for this group were considered reflective of the difference in mean body weight and not of toxicological significance. The exception to this pattern was the non-significantly increased mean absolute and relative weights of the testes for the high-dose group; although histopathologic evaluation did not reveal any morphologic abnormalities in this tissue.
The mean absolute and relative (to body and brain weight) ovarian weights for the high-dose females were markedly elevated, although differences from control were not statistically significant. These differences were due to a single animal, whose ovarian weight of 0.213 g was approximately six times the mean ovarian weight for the group when this value was excluded ($\bar{x}$ = 0.033 grams). Differences from control excluding this animal were not considered remarkable.
Other organ weight differences were attributed to body weight differences and were therefore not considered to be related to the administration of glyphosate.

Table B.6.5.16-6: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Group mean organ weights – oncogenicity study

Dose group	Nominal dose (ppm)	Organ weight (abs.)	Organ weight (rel. to body weight)	Organ weight (rel. to brain weight)
Testes (males)				
1 (control)	0	0.157 ± 0.056	4.97 ± 1.80	3.22 ± 1.17
2 (low)	1000	↓0.153 ± 0.058	↓4.71 ± 1.87	↓3.05 ± 1.18
3 (mid)	5000	↑0.158 ± 0.059	↑5.23 ± 2.10	↑3.26 ± 1.17
4 (high)	30000	↑0.168 ± 0.046 (+7%)	↑5.84 ± 1.58 (+18%)	↑3.46 ± 0.90
Ovaries (females)				
1 (control)	0	0.026 ± 0.034	9.40 ± 11.20	5.21 ± 7.26
2 (low)	1000	↓0.016 ± 0.010	↓5.46 ± 3.21	↓3.05 ± 1.92
3 (mid)	5000	↓0.021 ± 0.023	↓3.98 ± 7.45	↓4.35 ± 4.86
4 (high)	30000	↑0.041 ± 0.046	↑13.41 ± 14.81	↑8.17 ± 9.13
Brain (males)				
1 (control)	0	0.489 ± 0.023	1.55 ± 0.12	
2 (low)	1000	↑0.501 ± 0.033	↓1.54 ± 0.13	
3 (mid)	5000	↓0.480 ± 0.022	↑1.58 ± 0.15	
4 (high)	30000	↓0.486 ± 0.025	**↑1.69** ± 0.17**	
Brain (females)				
1 (control)	0	0.511 ± 0.044	2.01 ± 0.32	
2 (low)	1000	0.511 ± 0.026	↓1.83 ± 0.17	
3 (mid)	5000	↓0.502 ± 0.33	**↓1.66** ± 0.18**	
4 (high)	30000	↓0.501 ± 0.032	**↓1.69** ± 0.22**	
Heart (males)				
1 (control)	0	0.192 ± 0.026	6.11 ± 0.93	3.95 ± 0.57
2 (low)	1000	↓0.191 ± 0.024	↓5.87 ± 0.73	↓3.82 ± 0.48
3 (mid)	5000	↓0.186 ± 0.022	↑6.13 ± 0.77	↓3.88 ± 0.45
4 (high)	30000	↓0.175 ± 0.025	↓6.03 ± 0.65	↓3.60 ± 0.50
Heart (females)				
1 (control)	0	0.156 ± 0.042	6.02 ± 1.73	3.05 ±0.79
2 (low)	1000	↑0.164 ± 0.24	↓5.84 ± 0.53	↑3.22 ± 0.49
3 (mid)	5000	↑0.169 ± 0.048	↓5.56 ± 1.41	↑3.39 ± 0.49
4 (high)	30000	↓0.155 ± 0.017	**↓5.23[§§] ± 0.71**	↑3.12 ± 0.47
Kidneys (males)				
1 (control)	0	0.693 ± 0.144	2.19 ± 0.47	1.42 ± 0.30

Table B.6.5.16-6: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983): Group mean organ weights – oncogenicity study

Dose group	Nominal dose (ppm)	Organ weight (abs.)	Organ weight (rel. to body weight)	Organ weight (rel. to brain weight)
2 (low)	1000	↓0.682 ± 0.080	↓2.09 ± 0.22	↓1.36 ± 0.19
3 (mid)	5000	↓0.666 ± 0.130	↑2.21 ± 0.46	↓1.39 ± 0.27
4 (high)	30000	↓0.635 ± 0.098	↑2.20 ± 0.29	↓1.31 ± 0.19
Kidneys (females)				
1 (control)	0	0.489 ± 0.082	1.90 ± 0.27	9.57 ± 1.54
2 (low)	1000	↑0.495 ± 0.068	↓1.77 ± 0.23	↑9.68 ± 1.18
3 (mid)	5000	↑0.513 ± 0.088	**↓1.68* ± 0.24**	↑10.23 ± 1.74
4 (high)	30000	↑0.511 ± 0.078	↓1.71 ± 0.23	↑10.24 ± 1.70
Liver (males)				
1 (control)	0	1.753 ± 0.483	5.60 ± 1.80	3.59 ± 0.95
2 (low)	1000	↑1.822 ± 1.156	↑5.83 ± 3.79	↑3.80 ± 2.47
3 (mid)	5000	↓1.488 ± 0.179	↓4.88 ± 0.52	↓3.10 ± 0.37
4 (high)	30000	**↓1.475§ ± 0.319**	↓5.08 ± 0.95	↓3.03 ± 0.63
Liver (females)				
1 (control)	0	1.339 ± 0.316	5.12 ± 0.85	2.62 ± 0.61
2 (low)	1000	↑1.521 ± 0.401	↑5.37 ± 1.10	↑2.97 ± 0.74
3 (mid)	5000	↑1.595 ± 0.443	↑5.19 ± 1.19	↑3.18 ± 0.89
4 (high)	30000	↑1.393 ± 0.13	↓4.69 ± 0.83	↑2.80 ± 0.52

* Significantly different from control (Dunnett's test; $p \leq 0.05$); ** Significantly different from control (Dunnett's test; $p \leq 0.01$); §§ Significantly different from control (Dunn's Rank Sum; $p \leq 0.01$)

Histopathology

Non-neoplastic changes

A statistically significant increase in central lobular hepatocytes hypertrophy and necrosis was observed in high dose males. In addition, high dose males showed a significant increase in chronic interstitial nephritis in the kidney. An increase in atrophy zonal fasciculate of the adrenal glands was also observed in high dose males although this was not statistically significant.

In high dose females there was a significant increase in proximal tubule epithelia basophilia and hypertrophy in the kidney.

An increased frequency over controls was slight-to-mild epithelial hyperplasia of the urinary bladder in males. The incidence was 6%, 6%, 20% and 17% controls through high-dose, respectively. Whilst dose-response is not clearly shown and no positive trend was found according to the study report, the incidences are approximately three-fold higher than those of controls. Therefore, these increased frequency of epithelial hyperplasia of the urinary bladder in males is considered treatment-related.

All other tissue alterations occurred sporadically or were considered to have been spurious in their distribution. Most occurred with approximately equal frequency and severity in control and treated animals, and were judged to be unrelated to glyphosate administration.

Table B.6.5.16-7 A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983):: Summary of selected non-neoplastic findings

ppm	Males				Females			
	0	**1000**	**5000**	**30000**	**0**	**1000**	**5000**	**30000**
Liver; Central lobular hepatocyte hypertrophy	9 (18 %)	5 (10 %)	3 (6 %)	17 (34 %)[a]	3 (6 %)	5 (10%)	1 (2 %)	1 (2 %)

Table B.6.5.16-7 A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983):: Summary of selected non-neoplastic findings

ppm	Males				Females			
	0	**1000**	**5000**	**30000**	**0**	**1000**	**5000**	**30000**
Liver : Central lobular hepatocytes necrosis	0 (0%)	2 (4%)	2 (4%)	10 (20%)[a,b]	2 (4%)	1 (2%)	4 (8%)	2 (4%)
Kidneys ; Chronic interstitial nephritis (B/U)	5 (10 %)	2 (4 %)	7 (14 %)	12 (24 %)[a]	4 (8 %)	8 (16%)	2 (4 %)	4 (8 %)
Kidney proximal tubule epithelial basophilia and hypertrophy	15 (30%)	10 (20%)	15 (30%)	7 (14%)	0 (0%)	2 (4%)	4 (8%)	9 (18%)[a,b]
Adrenal glands ; Atrophy, zona fasciculate	13 (27 %)	9 (18 %)	9 (18 %)	19 (40 %)	1 (2 %)	0 (0 %)	0 (0 %)	0 (0 %)
Urinary bladder; epithelial hyperplasia	3 (6%)	3 (7%)	10 (21%)	8 (17%)	0 (0%)	0 (0%)	2 (4%)	0 (0%)

[a]Statistically significant linear trend ($p<0.01$) using the Cochran-Armitage test
[b] Statistically significant increase compared to control ($p<0.01$) using the Chi-Square test

Neoplastic findings
Neoplastic findings were of the type commonly encountered in mice. Bronchiolar-alveolar tumours of the lungs, hepatocellular neoplasms and tumours of the lymphoreticular system accounted for the majority of those encountered. There were no suspected test substance-associated trends in the incidence of these tumours or of any of the other spontaneously occurring neoplasms.

The only other neoplasms that occurred with any frequency in treated mice only, were renal tubule adenomas, which occurred in males. Three were present at the high-dose and one at the mid-dose level. The distribution of these benign tumours was considered spurious and unrelated to treatment in the study report due to the absence of other renal lesions suggestive of or supportive of an effect on the urinary system. The overall relevance of this finding in the context of the classification of glyphosate is included in Volume 1.

All other neoplasms occurred sporadically and were considered to have had no treatment relationship.

Table B.6.5.16-8: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983):: Summary of selected neoplastic findings

ppm	Males				Females			
	0	**1000**	**5000**	**30000**	**0**	**1000**	**5000**	**30000**
Kidneys; renal tubule adenoma	0 (0 %)	0 (0 %)	1 (2 %)	3 (6 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)

Table B.6.5.16-9: A chronic feeding study of glyphosate (Roundup® technical) in mice ([redacted] *et al.*, 1983):: Summary of malignant tumours in the lymphoreticular system

Type of tumour	Sex	Control	Low	Mid	High dose
Dose level		**0**	**1000 ppm**	**5000 ppm**	**30000 ppm**
Lymphoblastic lymphosarcoma with leukaemia	M	1	4	3	2
Lymphoblastic lymphosarcoma without leukaemia	M	0	1	0	0
Composite lymphosarcoma	M	1	0	1	0
Lymphoreticular neoplasms (total)	**M**	**2/48**	**5/49**	**4/50**	**2/49**
Lymphoblastic lymphosarcoma with leukaemia	F	1	4	5	1
Lymphoblastic lymphosarcoma without leukaemia	F	0	1	0	3
Composite lymphosarcoma	F	4	1	1	6
Granulocytic leukaemia [a]	F	0	3	0	0
Lymphoreticular neoplasms (total)	F	5/49	9/49	6/49	10/49

[a] it should be noted that granulocytic leukaemia are not lymphomas.

Assessment and conclusion by applicant: In conclusion, glyphosate technical was not carcinogenic in male and female CD-1 mice following continuous dietary exposure of up to 30000 ppm (equivalent to 4841 mg/kg bw/day for males and 5874 mg/kg bw/day for females) for 104 weeks. Based on non-neoplastic histological changes affecting urinary bladder epithelium in male mice at 5000 ppm glyphosate in diet (814 mg/kg bw/day) and higher, the chronic mouse NOAEL is considered the low dose of 1000 ppm (157 mg/kg bw/day).

Assessment and conclusion by RMS:
The RMS agrees with the proposed NOAEL of 1000 ppm (157 mg/kg bw/day). This is based on an increased frequency of epithelial hyperplasia in the urinary bladder of males dosed at 5000 ppm and above.
At 3000 ppm a reduced body weight (>10%), increased absolute and relative testis weight, hepatocyte hypertrophy and necrosis, chronic interstitial nephritis in kidney and kidney renal tubule adenoma was observed.

B.6.5.17. Long-term toxicity – mouse, study 7

Data point:	CA 5.5/024
Report author	[redacted]
Report year	1982 (original report) 1992 (revised translated version)
Report title	18-Month Carcinogenicity Study of Glyphosate in Mice
Report No	8010
Document No	Not reported
Guidelines followed in study	No guideline followed; similar to OECD 451 (1981)
Deviations from current test guideline (OECD 451, 2018)	The following deviations from the current test guideline were noted : - No data on test item purity provided; - Animal weight at dosing, acclimatisation period and diet were reported; - No in-life dates were reported; - Only two dose levels were tested; - Body weight were measured only monthly;

	- The number of animals surviving up to scheduled termination and subjected to pathological examination was too small for meaningful evaluation; - The mice which had died inter-currently were not examined and the cause of death is unknown; - Histopathology was performed without determining cervix, coagulating glands, harderian gland, lacrimal gland, pituitary, thymus and vagina.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP)
Acceptability/Reliability:	Conclusion applicants : Invalid (Category 3b) Due to several limitations the study is not considered acceptable for hazard and risk assessment. Conclusion AGG: Considering the severe deviations noted the study is concluded to be unacceptable. The dose levels are also noted to be very low compared to the previous carcinogenicity studies.

Glyphosate (Technical) was administered to groups of 50 male and female CFLP mice (bred in a facility in [redacted] 26 - 30 days old at study initiation) per dose at dietary levels of 0, 100 and 300 ppm (equivalent to 0, 12.6 and 37.7 mg/kg bw/day for males and 0, 16.3 and 44.5 mg/kg bw/day for females). The administration period was 18 months.

Animals were kept under continuous observation for behaviour, general condition and lethality. External examinations were performed periodically. Body weight gains were measured monthly and food consumption was measured weekly. All surviving animals were necropsied after the treatment period and examined for gross pathological and histopathological findings.

No toxic symptoms were noted during the study regarding body weight changes and food consumption. Mortality was high in all groups (11-23 of 50 mice). In-life, necropsy and histopathology data excluded the dose-related toxicity of the test item at the applied dose levels. The number of tumours detected partly by gross pathology and identified by histopathology showed a similar distribution in treated and control groups.

This was the case with the number of tumour bearing animals, too. The various kinds of tumours showed a certain difference in their occurrence in treated and control groups without convincing and statistically significant signed of dose dependence. Consequently, the available study data were against the carcinogenicity of glyphosate in mice in the given experimental conditions.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Glyphosate
Description:	No data given in the report
Lot/Batch #:	14/980-03090380
Purity:	No data given in the report
Stability of test compound:	No data given in the report

2. **Vehicle and/or positive control:** Diet / none
3. **Test animals:**

Species:	Mouse

Strain:	CFLP	
Source:	[redacted]	
Age:	28 ± 2 days	
Sex:	Males and females	
Weight at dosing:	No data given in the report	
Acclimation period:	No data given in the report	
Diet/Food:	No data given in the report	
Water:	Tap water, *ad libitum*	
Housing:	Groups of 10 per sex in OMKER III shoebox type cages	
Environmental conditions:	Temperature:	21 ± 3 °C
	Humidity:	50 ± 5 %
	Air changes:	10 times/hour

B: Study design and methods

In life dates: Not reported, but issued in 1982

Animal assignment and treatment:
In a carcinogenicity study groups of 50 CFLP mice per sex received daily dietary doses of 0, 100 or 300 ppm (equivalent to 0, 12.6 or 37.7 mg/kg bw/day for males and 0, 16.3 or 44.5 mg/kg bw/day for females) glyphosate for 18 month.

Clinical observations
The study report claims that the animals were kept under continuous observation for behaviour, general condition and lethality although this is considered unlikely by the RMS. External examinations were performed periodically.

Body weight
Individual body weights were recorded on monthly.

Food consumption
Food consumption was recorded once weekly.

Sacrifice and pathology
Necropsy was performed on all animals that died during the observation period and all animals at scheduled termination.

Tissue samples for histopathology were taken from the following organs of all animals: adrenals, aorta, bone with bone marrow, brain, duodenum, epididymides, eyes with optic nerve, gall bladder, heart, ileum, jejunum, large intestine, kidneys, lungs, lymph nodes (mesenteric and submandibular), muscle (femoral), oesophagus, ovaries, pancreas, prostate, salivary glands, sciatic nerve, seminal vesicles, skin with mammary tissue, spleen, spinal cord, stomach, testes, trachea, thyroid, tongue, tumours, urinary bladder and uterus.

Statistics
For analysing the incidence of tumours and bearing animals χ^2 test was used.

RESULTS

A. ANALYSIS/PREPARATION OF DOSE FORMULATIONS
One week before study initiation, than monthly, three different pre-mixes were prepared from glyphosate substance and soya. Premixes were submitted monthly to LATI where they were used for diet preparation. The prepared food samples were given to the animals for one month.

B. MORTALITY

The numbers of pre-terminal deaths in the main group are displayed in Table :

Table B.6.5.17-1: 18-Month Carcinogenicity Study of Glyphosate in Mice [redacted], 1982/1992): Mortality

Sex	Dose group [ppm]					
	0		100		300	
	Males	Females	Males	Females	Males	Females
Month 1 – 3	0	1	1	0	0	0
Month 4 – 6	0	2	1	7	0	2
Month 7 – 9	2	7	7	7	3	2
Month 10 – 12	3	7	15	5	5	7
Month 13 – 15	21	7	4	6	4	13
Month 16 – 18	13	12	8	9	15	12
Combined	39/50	36/50	36/50	34/50	27/50	36/50

Both the time dependent incidence and the cumulative values of deaths excluded the negative influence of test substance and dose on the life expectance of mice. The males of the 100 ppm dose group died somewhat earlier, while the survival rate was the highest in the 300 ppm male group with 25/50 overall mortality. Conclusively, test substance exerted no impairment on the health condition of mice as expressed by the mortality values.

C. CLINICAL OBSERVATIONS

Mice had a symptom-free general health condition during the study.

D. BODY WEIGHT

The kinetics of body weight change were of similar pattern in the treated and control groups. Maximal weights were measured mostly between month 6-12 with a slight declination by the end of the study period. The rate of decrease was slightly stronger in 100 and 300 ppm males, but was milder than in the corresponding control, in 100 ppm females.

Table B.6.5.17-2: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Group mean body weights

Time point	Sex	Males			Females		
	Dose [ppm]	0	100	300	0	100	300
	No. of rats	50	50	50	50	50	50
Month 1		29.5	↑29.7	29.5	24.4	↑24.6	↑25.1
Month 2		43.2	↓41.4	↓41.0	33.1	↑33.6	↓28.0
Month 3		47.4	↓45.9	↓46.5	35.5	↑36.7	↑37.1
Month 4		51.8	↓49.8	↓51.0	35.9	↑40.0	↑41.2
Month 5		53.6	↓51.4	↓51.3	38.0	↑39.8	↑41.8
Month 6		55.3	↑55.4	↑53.8	40.4	↓40.3	↑44.3
Month 7		59.7	↓57.4	↓57.3	42.3	42.3	↑46.8
Month 8		61.4	↓59.6	↓58.8	42.7	↓41.1	↑47.3
Month 9		64.1	↓62.4	↓60.3	46.2	↓45.4	↑51.5
Month 10		62.8	↑67.8	↓60.2	46.4	↑47.6	↑52.9
Month 11		64.7	↓64.5	↓57.9	48.2	↓47.7	↑55.7
Month 12		62.8	↓54.8	↓57.7	45.0	↑46.9	↑49.3
Month 13		54.1	↓53.1	↑55.2	42.9	↑46.0	↑45.2
Month 14		49.4	↑52.9	↑55.5	42.3	↑47.6	↑44.1
Month 15		47.9	↑51.1	↑51.6	41.7	↑46.8	↑44.7
Month 16		47.5	↑50.5	↑51.2	43.4	↑46.4	↑43.5

Table B.6.5.17-2: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted] 1982/1992): Group mean body weights

Time point	Sex	Males			Females		
	Dose [ppm]	0	100	300	0	100	300
	No. of rats	50	50	50	50	50	50
Month 17		48.0	↓46.2	↑48.6	45.0	↑45.3	↓44.0

E. FOOD CONSUMPTION AND COMPOUND INTAKE

No effect on food consumption occurred. The group mean achieved doses are summarised below.

Table B.6.5.17-2: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Group mean achieved dose levels

Dose group	Dietary concentration [ppm]	Mean achieved dose level [mg/kg bw/day]	
		Males	Females
low	100	12.6	16.3
high	300	37.7	44.5

F. NECROPSY

At terminal sacrifice necropsy findings referred first of all to regular or irregular enlargements in (liver, lung, lymph nodes, uterus and ovary) or on the surface of some organs, like intestines (M+F). Since no distinction was made in respect of their nature (tumor, inflammation, cyst or functional status), therefor, the seemingly dose-related incidence of alterations might refer to, but do not evidence the real treatment dependence of any of the lesions. The study report mentions that the observed enlargements were partly related to inflammatory-parasitic processes. They were related occasionally to the physiological state of organ, like enlarged Peyer's plaques appearing as focal enlargements in or on the small intestines.

Table B.6.5.17-3: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted] 1982/1992): Group mean food consumption

Parameter	Sex	Males/Females		
	Dose [ppm]	0	100	300
	No. of rats	25	30	37
Liver: Focal enlargement		3 (12)	3 (10)	8 (21)
Spleen: Enlargement		2 (8)	2 (7)	6 (16)
Small intestines: round shaped formation		3 (12)	4 (13)	8 (21)
Lymph nodes: Enlargement		6 (24)	5 (17)	6 (16)
Lung: Focal enlargement		7 (28)	6 (20)	9 (24)

() Number in parenthesis are % of the respective group/parameter

Histopathology

Non-neoplastic changes

There was no treatment related effect on non-neoplastic findings.

Neoplastic changes
The incidence of tumour findings are summarised in the following table:

Table B.6.5.17-4: 18-Month Carcinogenicity Study of Glyphosate in Mice ([redacted], 1982/1992): Neoplastic lesions

Parameter	Sex	Males			Females		
	Dose [ppm]	0	100	300	0	100	300
	No. of rats						
Liver haematoma		1/11	0/14	5/23	0/14	0/16	1/14
Pulmunorary adenoma, carcinoma		1/11	1/14	4/23	4/14	4/16	2/14
Malignant lymphoma		1/11	1/14	1/23	1/14	2/16	1/14

Assessment and conclusion by applicant:
Glyphosate did not show any significant increase in tumour formation or toxicological effects when fed to CFLP mice up to the highest tested dose of 300 ppm (dietary admix) for 18 months. Consequently the available study data do not reveal the carcinogenicity of glyphosate in mice in the given experimental conditions. The NOAEL in this study is 300 ppm (equivalent to 37.7 mg/kg bw/day for males and 44.5 mg/kg bw/day for females).

Due to several limitations the study is not considered acceptable for hazard and risk assessment.

Assessment and conclusion by RMS:
The study showed a wide number of limitations including dose levels being too low (max 300 ppm), only two dose levels being tested, lack of detail on test material and animals, body weight measured only monthly, no pathological examination on animals that died or were sacrificed during the study and the number of animals at termination being too low (11 to 23). Based on these limitations the study was concluded to be unacceptable.

B.6.5.18. Long-term toxicity – public literature

Epidemiological studies:

Refer to Volume 1 for an overall consideration of the epidemiological studies.

During the last evaluation in 2015, a number of epidemiology studies were evaluated and summaries provided within the dossier. The summaries of these studies (beyond the scope of a 10 year literature search) were additionally included in this dossier in order to allow a comprehensive evaluation. All studies were re-evaluated by the current RMS as indicated in the study summaries in Section B.6.5.8.13 to B.6.5.18.26.

Other public literature:

Refer to Volume 1 for an overall consideration of the other public literature studies.

B.6.5.18.1. Supporting publications – Crump, 2020

Data point:	CA 5.5/026
Report author	Crump, K. et al.

Report year	2020
Report title	Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate.
Document No	https://doi.org/10.1093/toxsci/kfaa039
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previously submitted	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG : -/Reliable without restrictions Conclusion AGG : Reliable.

Full summary of the study according to OECD format

Abstract

Glyphosate is a widely used herbicide worldwide. In 2015, the International Agency for Research on Cancer (IARC) reviewed glyphosate cancer bioassays and human studies and declared that the evidence for carcinogenicity of glyphosate is sufficient in experimental animals. The authors analyzed ten glyphosate rodent bioassays, including those in which IARC found evidence of carcinogenicity, using a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities. The test statistics for these permutation tests are functions of p-values from a standard test for dose-response trend applied to each specific type of tumor. The authors evaluated three permutation tests, using as test statistics the smallest p-value from a standard statistical test for dose-response trend and the number of such tests for which the p-value is less than or equal to 0.05 or 0.01. The false-positive probabilities obtained from two implementations of these three permutation tests are: smallest p-value: 0.26, 0.17, p-values ≤ 0.05: 0.08, 0.12, p-values ≤ 0.01: 0.06, 0.08. In addition, the authors found more evidence for negative dose-response trends than positive. Thus, the authors found no strong evidence that glyphosate is an animal carcinogen. The main cause for the discrepancy between IARC's finding and ours appears to be that IARC did not account for the large number of tumor responses analyzed and the increased likelihood that several of these would show statistical significance simply by chance. This work provides a more comprehensive analysis of the animal carcinogenicity data for this important herbicide than previously available.

Materials and methods

Analysis of bioassay data - Of the bioassays identified by U.S. EPA, EFSA, or IARC, 10 glyphosate cancer studies of sufficient quality were selected which allowed the analysis of individual animal data. The characteristics of the selected studies are summarized in Table B.6.5.18.1-1. Three studies were excluded as they concerned formulation studies, two for having incomplete data and one due to a viral outbreak during the study.

Table B.6.5.18.1-1: Characteristics of the bioassays selected for analysis.

Bioassay	Species	Strain	# Dose Groups/ Sex	Animals/Dose	Maximum Dose[a] (mg/kg/d)		Maximum Weeks on test	Sites Where Histopathology was Conducted in All Dose Groups[b]
					Males	Females		
[redacted]et al 1993b[c]	Mouse	CD-1	4	50	988	1000	105	Kidney, Liver, Lung, Vascular System
[redacted]1983[c]	Mouse	CD-1	4	50	4841	5873	102	(all)
[redacted]et al 2009b	Mouse	CD-1	4	51	810	1081	81	Kidney, Liver and Lung
[redacted]1997	Mouse	CD-1-ICR	4	50	4348	4116	78	(all)
[redacted]et al 1993a[c]	Rat	SD[d]	5	50	1007	1018	105	Kidney, Liver, Lung and Salivary Glands: Parotid, Mandibular and Sublingual
[redacted]1981[c]	Rat	SD	4	50	31.49	34.02	111	(all)
[redacted]1990[c]	Rat	SD	4	60	940	1183	105	(all)
[redacted]2001[c]	Rat	Wistar	4	64	1214	1498	104	(all)
[redacted]1996	Rat	Wistar	4	50	595.2	886	107	(none)
[redacted]et al 2009a	Rat	Wistar	4	51	1077	1382	105	Kidney, Liver, Lung and Bone Marrow

[a] All doses in each bioassay are listed in Table 2.
[b] Systemic tumors are assumed to have been searched for if at least one tissue in an animal was given a histopathological examination.
[c] These six studies were evaluated by IARC. IARC (2015) also reviewed two additional studies in which they identified shortcomings, but which they did not claim were "inadequate": Chruscielska et al (2000) and JMPR (2006). No glyphosate-related tumor responses were noted in either of these studies.
[d] SD = Sprague Dawley.

Eight of the 10 studies were assigned a Klimisch code 1 (reliable without restrictions). The [redacted] (1983) study was assigned a Klimisch code 2 (reliable with restrictions) mainly because this study was initiated before the implementation of the OECD test guidelines and GLP, and the study of [redacted] (1981) also a pre-GLP and pre-guideline study was assigned a Klimisch code 3 (not reliable) mainly because of the low power of the study due to the low dose range selected.

Statistical tests - The statistical tests applied in the analysis were functions of p-values obtained from conventional continuity-corrected poly-3 tests (survival-adjusted Cochran-Armitage test) for trend applied to each type of tumor or combination of tumor types in each bioassay. In the present analysis, the continuity-corrected version of the poly-3 test used was copied from a key portion of the computer program used by the NTP. Direct comparisons have shown that the author's implementation gives the same results as the version used by the NTP. Throughout this paper, all implementations of the poly-3 test, are one-sided, as are the NTP implementations of the test.

Results from 3 multi-response permutation tests are presented. In the simplest test, referred to as the "min-test", the test statistic is the smallest p-value obtained from applying the poly-3 test to all tumor types in all of the 10 bioassays. In the simplest implementation of this test, animals are randomly reassigned to dose groups (permuted among dose groups) in a Monte Carlo analysis, keeping the total numbers of animals in each dose group equal to the number in the original data. The tumors in each such reassignment are analyzed using the poly-3 test in exactly the same way as in the original data. Males and females are permuted separately. The false positive rate is the proportion of random reassignments that result in a smallest poly-3 p-value that is smaller than or equal to the smallest poly-3 p-value obtained from the original data.
In addition to the min-test, two additional permutation tests were computed. The test statistics for these tests were the number of poly-3 tests of tumors in the original data for which the p-value is less than or equal to the critical value of 0.05 (05-test) or 0.01 (01-test). The false positive rates for these tests are the proportion of random permutations of the data for which the number of poly-3 p-values from the permuted data that are less than or equal to the critical value equal or exceed the number from the original data. The min-test could have enhanced power in a situation in which a test agent causes cancer at a single site, whereas the 05-test could have enhanced power when a test agent causes detectable cancer of several types. The permutation tests described above are conditional, not just on the total number of tumors, but also on the patterns of tumors occurring in individual animals.

In addition to conducting conventional poly-3 tests on specific types of tumors, tests were also conducted on combinations of tumor types thought to have a common origin such as liver adenomas and carcinomas. Since including these combinations resulted in the same tumors being present in multiple analyses, it was decided to perform two analyses, one (primary analysis) that included all of the individual tumors and combinations, and one (reduced analysis) in which individual tumors and combinations of tumors were removed from the analysis if they were part of a more inclusive tumor combination (e.g. the individual tumor categories of liver adenoma and liver carcinoma were removed and only the combination was used in the analysis).

For 5 of the glyphosate bioassays, all tissues listed for histopathological examination were scheduled for a histological examination in all animals of all dose groups. In the remaining 5 bioassays, control and high dose animals were all given a complete histopathological examination, along with the animals that died before the final sacrifice in the intermediate dose groups. In addition, certain tissues in all animals were scheduled for a histopathological examination regardless of when they died.

Simple randomization suffers from a potential bias due to dose-related differential survival, and, for studies with incomplete histopathology, a problem of data comparability.

In each of the 10 bioassays, dose-related effects on survival were tested using a Cochran-Armitage test for negative trend on the proportions of animals surviving to final sacrifice in the various dose groups. Regardless of the outcome of this test, to control for potential dose-related differences in survival each randomization of the data maintained the same number of survivors and non-survivors in each group as was seen in the actual data.

For other tissues than the mandatory tissues in the studies with incomplete histopathology, only the non-survivors in the intermediate dosed groups could be used in the trend analyses. For mandatory tissues, the survivors and non-survivors were separately permuted. Mandatory tissues and other tissues had to be separately randomized in studies with incomplete histopathology to make sure that all pathological information routinely collected in this type of studies is included.

In all applications of the poly-3 test, the test is applied only to data from one sex in a single study and the p-values from the poly-3 tests of all the studies are combined to create the "global" tests (min-test, 05-test and 01-test) to give the correct false positive rates. In addition to the randomization procedures for testing for positive dose-response trends in tumor incidence, the same procedures were repeated after reconfiguring the poly-3 test for negative trends.

Results

When the frequency of poly-3 p-values for positive trend computed from all tumors in all 10 bioassays in which at least two tumors occurred are considered there is an excess of large p-values (close to 1.0) compared to small p-values (close to 0.0). Since the version of the poly-3 trend test applied is a one-sided test for a positive trend, p-values close to 1.0 would translate into p-values near 0.0 for one-sided trend tests for anti-carcinogenicity. However, this is not necessarily evidence that glyphosate is anti-carcinogenic.

Results of tests for a dose-related decrease in survival in each study show that in none of the bioassays analyzed this test was statistically significant. Moreover, 4 of the datasets had p-values in excess of 0.95 which indicates a significant positive trend in survival with increasing dose.

The 24 tumors for which the poly-3 test for a positive dose-related trend was significant at the 0.05 level in the primary analysis are shown in Table B.6.5.18.1-2. Pancreatic islet-cell adenoma in male rats reported in the [redacted] (1990) study, which had responses of 1/58, 8/57, 5/60 and 7/59 is not

listed because it did not have a significant dose-related trend. In an identical analysis with the poly-3 test configured to test for a negative dose-related trend, there were 26 tumors for which the dose-response trend was significantly negative at the 0.05 level.

Table B.6.5.18.1-2: Tumors with a significant positive trend (poly-3 p ≤ 0.05)

Bioassay	Species/ Sex	Tumor	Summary Tumor Incidence					Poly-3 p-value	Cited by IARC[a]
[redacted]et al. 1993b	M/M	Haemangiosarcoma	0/50	0/50	0/50	4/50		0.0013	IARC
[redacted]1981	R/F	Thyroid: C-cell Carcinoma	1/47	0/49	2/50	6/47		0.0015	
[redacted]1997	M/F	Hemangioma	0/50	0/50	2/50	5/50		0.0028	
[redacted]1997	M/F	Hemangioma, Hemangiosarcoma	0/50	0/50	3/50	5/50		0.0062	
[redacted]1990	R/F	Adrenal: Cortical Carcinoma	0/60	0/60	0/60	3/60		0.0072	
[redacted]1997	M/F	Osteoma, Osteosarcoma	0/50	0/50	0/50	3/50		0.0074	
[redacted]l. 2009b	M/M	Lymphoma	0/51	1/51	2/51	5/51		0.0076	
[redacted]2001	R/M	Liver: Hepatocellular Adenoma	0/64	2/64	0/64	5/64		0.014	
[redacted]1981	R/M	Testis: Interstitial Cell Tumor	0/50	3/50	1/50	6/50		0.021	
[redacted]1990	R/M	Liver: Hepatocellular Adenoma	3/60	2/60	3/60	8/60		0.022	IARC[b]
[redacted]t al. 1993a	R/F	Lipoma	0/50	0/50	0/50	0/50	2/50	0.022	
[redacted]l. 2009b	M/M	Lung: Adenocarcinoma	5/50	5/51	7/51	11/51		0.025	
[redacted]1983	M/M	Kidneys: Renal Tubal Adenoma	0/49	0/49	1/50	3/50		0.034	IARC
[redacted]981	R/F	Lipoma	0/50	0/50	0/50	2/50		0.036	
[redacted]1997	M/M	Malignant Lymphoma	2/50	3/50	0/50	6/50		0.038	
[redacted]1983	M/F	Lymphoblastic Lymphosarcoma	0/50	1/50	0/50	3/50		0.041	
[redacted]1997	M/F	Osteosarcoma	0/50	0/50	0/50	2/50		0.041	
[redacted]1997	M/M	Kidney: Adenoma	0/50	0/50	0/50	2/50		0.042	
[redacted]1997	M/M	Hemangiosarcoma	0/50	0/50	0/50	2/50		0.043	
[redacted]990	R/M	Neurofibroma, Neurofibrocarcinoma	0/60	0/60	0/60	2/60		0.045	
[redacted]1997	M/F	Harderian Gland: Adenoma	1/50	3/50	0/50	5/50		0.046	
[redacted]1990	R/F	Thyroid Gland: C-cell Adenoma	2/60	2/60	6/60	6/60		0.047	IARC
[redacted]996	R/M	Lymphoma	0/50	0/50	0/50	2/50		0.049	
[redacted]1990	R/F	Thyroid Gland: C-cell Adenoma or Carcinoma	2/60	2/60	7/60	6/60		0.049	

[a] Indicates tumor responses cited by IARC (2015) as evidence of carcinogenicity. Pancretic islets in male rats in [redacted] (1990) was also cited by IARC (1/58, 8/57, 5/60 and 7/59) but this response did not give a p-value ≤ 0.05 by the Poly-3 trend test.

[b] IARC (2015) resported tumor responses of 2, 2, 3, 7.

Table B.6.5.18.1-3 shows the results for the three permutation tests for positive trend, both for the primary analysis and the reduced analysis. The most significant poly-3 trend in all 10 bioassays was for hemangiosarcoma in male mice in the [redacted] *et al*. (1993) study with a p-value of 0.0013. The actual significance of this smallest p-value, which is the false positive rate for the min-test, was 0.26 based on the primary analysis, rather than the naive value of 0.0013. This means that 26 % of the randomizations of the 10 datasets gave a smallest p-value less than or equal to the smallest p-value obtained from the original data.

The false positive rate for the 05-test was 0.08, which means that 8 % of randomizations of the 10 datasets found at least 24 sites for which the poly-3 p-value was ≤ 0.05. The results from all permutation tests based on the reduced data were similar to those based on the primary data. The false positive rate for the 01-test was 0.06 in the primary analysis and 0.08 in the reduced analysis. Overall, these findings suggest that, after accounting for the number of statistical tests performed, there was no clear evidence of a positive dose-related trend in tumor occurrence.

Table B.6.5.18.1-3: Results of multi-response permutation test for positive dose-related trends in tumor occurrence

	Primary Analysis		Reduced Analysis[a]	
Description of Test	Test Statistic[b]	Statistical Significance of Test Statistic[c]	Test Statistic[b]	Statistical Significance of Test Statistic[c]
Min Test	p = 0.0013	p = 0.26	p = 0.0013	p = 0.17
05 Test	24	p = 0.08	14	p = 0.12
01 Test	7	p = 0.06	4	p = 0.08
Number of trend tests	525		304	

[a]The reduced analysis removed from the analysis tumors and combinations of tumors that were included in larger combinations.

[b]The test statistic of the min test is the smallest poly-3 p-value obtained from any tumor in any study in the original data. The test statistics of the 05 test and the 01 test are the number of tumors for which the poly-3 p-value was ≤ 0.05 or ≤ 0.01, respectively.

[c]Calculated using 5,000 simulations.

The evidence for negative trends is greater than that for positive trends in all analyses. The smallest poly-3 p-value for a negative trend was 0.0008 for bronchiolar-alveolar adenoma in female mice in [redacted] (1983), whereas the smallest p-value for a positive trend was 0.0013. The 01-test for a negative trend was highly significant in both the primary and reduced analyses with a p-value of 0.002 for each. These findings suggest stronger evidence for negative rather than positive dose-response trends in tumor occurrence.

Discussion and conclusions

The highest doses given to any animal groups in the 10 bioassays analyzed were 5,873 mg/kg bw/day and 4,841 mg/kg bw/day in female and male mice, respectively. Despite the extremely high doses, there was no evidence of reduced survival in this study. On the contrary, there was a statistically significantly enhanced survival in male mice in this study, as well as in male animals in several other bioassays. The use of the individual animal data allowed the authors to distinguish between an adenoma and a carcinoma occurring in separate animals and both tumors occurring together in a single animal. Knowledge of the age at death of each individual animal is required for the conduct of the poly-3 test. In addition to the application of the poly-3 test, which is an age-adjusted Cochran-Armitage test, age was also controlled by keeping the numbers of animals surviving to final sacrifice in each dose group the same in all permutations as in the original data.

In the primary analysis of all bioassays 525 poly-3 analyses were conducted of individual tumor responses, of which a total of 174 were on combinations of individual tumor types that may have similar etiologies. In the primary analysis, individual tumors can appear in more than one poly-3 analysis. Since this will happen in the original data and the permuted data with equal frequency, it will not bias the analysis. Also a reduced analysis was conducted in which individual tumors and combinations of tumors were removed from the analysis if they were part of a more inclusive tumor combination. This reduced analysis involved 304 poly-3 analyses. Results from these two analyses were quite similar.

The smallest poly-3 p-value (0.0013) found in the analysis of all the datasets was that for hemangiosarcoma in male mice in the [redacted] *et al*. study. The analysis showed that the actual false positive rate for this finding after accounting for multiple comparisons was 0.26 in the primary analysis and 0.17 in the reduced analysis. Neither the 05-test nor the 01-test gave a false positive rate that was clearly less than 0.05 in the primary and reduced analysis, although the false positive rate for the 01 test in the primary analysis was near the boundary of 0.05. The statistically significant (p = 0.0013) response of 8 % in CD-1 males in the [redacted] *et al*. study resulted from 4 hemangiosarcomas at a dose level of 1,000 mg/kg bw/day, with no hemangiomas or hemangiosarcomas reported at the 3 lower doses. The

CD-1 male mice in the [redacted] study were exposed to 4,831 mg/kg bw/day, a dose nearly 5 times that used in the [redacted] *et al*. study with no hemangiomas or hemangiosarcomas. Moreover, the incidence of 8% was still within the historical control range reported for male CD-1 mice by [redacted] *et al*. (0-8%) and [redacted] (2005) (0-12%). The 10% incidence in hemangioma/ hemangiosarcoma in female CD-1 mice reported in the [redacted] (1997) study is within the historical control range of 0-12% of hemangiosarcoma reported by [redacted] (2005). The lack of a consistent dose response in either males or females suggests that finding significant responses in hemangioma and hemangiosarcoma in both sexes of mice may be attributable to chance, especially considering that this represents the "worst case" of more than 100 tumor sites/types in these bioassays that could have shown evidence of carcinogenicity.

The only other tumor in the mouse studies that the IARC regarded as being clearly related to glyphosate exposure was the marginally significant increase (0/49, 0/49, 1/50, 3/50) in kidney adenoma in male mice observed in the [redacted] study. However, additional step sectioning of kidneys in the dosed and control groups revealed one kidney adenoma in the control group, but no additional kidney tumors in the dosed groups. Since the new data provided did not identify this control animal, this additional tumor could not be taken into account in the analysis of this paper. When this tumor-bearing control animal is taken into account in the trend analysis the trend is not anymore statistically significant, adding to the evidence that the tumor increases reported in the glyphosate studies are due to chance.

Comparing the results of negative dose-related trends with those testing for a positive trend, the evidence for an effect was stronger for negative than for positive trends. The smallest p-value for a positive trend was 0.0013 versus 0.0008 for a negative trend, although the corresponding false positive rates after correcting for multiple comparisons were 0.26 and 0.11 demonstrating how adjusting for multiple comparisons can change the interpretation of analyses of individual tumors. The only clearly significant results for any of the 3 permutation tests were the highly significant 01-tests for negative trend in both the primary and reduced analysis. The authors however question against assuming that this finding is evidence of an anti-carcinogenic effect as there may be other explanations, such as an effect on reduced body weights and an effect on food palatability at high doses as it is known that food restriction can lead to lower cancer incidences.

In all 10 bioassays, the analysis made in this paper identified 24 tumors that exhibited a poly-3 positive trend with a p-value ≤ 0.05. Nevertheless, after accounting for the multitude of statistical tests this analysis did not find that number statistically significant ($p = 0.08$). The statistical analysis of 10 glyphosate bioassays presented in this paper found no strong statistical evidence that glyphosate is carcinogenic. The main cause for the discrepancy between the analysis made by IARC and that of the authors appears to be that IARC failed to consider the large number of statistical tests performed in the multiple bioassays they reviewed and the resulting multiple comparison problem. IARC and other organizations involved with interpreting results from large data sets to which a large number of statistical tests have been applied should consider applying analyses of the type used in this paper to make informed and reasonable decisions. The present analysis provides new information on the potential carcinogenicity of glyphosate by being the first to provide results from statistical tests with correct false positive rates. These tests found no strong or convincing evidence that glyphosate is an animal carcinogen.

Assessment and conclusion by applicant:

Ten cancer bioassays of sufficient quality and which allowed the analysis of individual animal data was selected for the application of a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities. The statistical tests applied in the analysis were functions of p-values obtained from conventional continuity-corrected poly-3 tests for trend applied to each type of tumor or combination of tumor types in each bioassay. Results from 3 multi-response permutation tests are reported and discussed: the "min-test", the "05-test" and the "01-test". In the min-test, the test statistic is the smallest p-value obtained from applying the poly-3 test to all tumor types in all bioassays

investigated. Animals are randomly reassigned to dose groups in a Monte Carlo analysis, keeping the total numbers of animals in each dose group equal to the number in the original data. The tumors in each such reassignment are analyzed using the poly-3 test in exactly the same way as in the original data. Males and females are permuted separately. The false positive rate is the proportion of random reassignments that result in a smallest poly-3 p-value that is smaller than or equal to the smallest poly-3 p-value obtained from the original data. The test statistics for the 05-test and the 01-test are the number of poly-3 tests of tumors in the original data for which the p-value is less than or equal to the critical value of 0.05 or 0.01, respectively. In all applications of the poly-3 test, the test is applied only to data from one sex in a single study and the p-values from the poly-3 tests of all the studies are combined to create the "global" tests (min-test, 05-test and 01-test) to give the correct false positive rates. In addition to the randomization procedures for testing for positive dose-response trends in tumor incidence, the same procedures were repeated after reconfiguring the poly-3 test for negative trends. When the frequency of poly-3 p-values for positive trend computed from all tumors in all 10 bioassays in which at least two tumors occurred are considered there is an excess of large p-values (close to 1.0) compared to small p-values (close to 0.0). Since the version of the poly-3 trend test applied is a one-sided test for a positive trend, p-values close to 1.0 would translate into p-values near 0.0 for one-sided trend tests for anti-carcinogenicity. Results of tests for a dose-related decrease in survival in each study show that in none of the bioassays analyzed this test was statistically significant. Moreover, 4 of the datasets had p-values in excess of 0.95 which indicates a significant positive trend in survival with increasing dose. The most significant poly-3 trend in all 10 bioassays was found in the [redacted] *et al.* (1993) study for hemangiosarcoma in male mice with a p-value of 0.0013. The actual significance of this smallest p-value, which is the false positive rate for the min-test, was 0.26 based on the primary analysis, rather than the naive value of 0.0013. This means that 26 % of the randomizations of the 10 datasets gave a smallest p-value less than or equal to the smallest p-value obtained from the original data. Besides, the incidence in hemangiosarcomas (8 %) remained within the historical control range and no such tumors were identified in another mouse study at a dose level nearly 5 times of that used in the [redacted] *et al.* (1993) study. Overall, these findings suggest that, after accounting for the number of statistical tests performed, there was no clear evidence of a positive dose-related trend in tumor occurrence. The 01-test for a negative trend was highly significant with a p-value of 0.002. These findings suggest stronger evidence for negative rather than positive dose-response trends in tumor occurrence. In all 10 bioassays investigated, the analysis made in this paper identified 24 tumors that exhibited a poly-3 positive trend with a p-value of less than or equal to 0.05. Nevertheless, after accounting for the multitude of statistical tests this analysis did not find that number statistically significant ($p = 0.08$). The statistical analysis of 10 glyphosate bioassays presented in this paper found no strong statistical evidence that glyphosate is carcinogenic. This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.

Reliability criteria for *in vivo* toxicology studies made by the applicant

Publication: Crump *et al*., 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N.A.	
Study completely described and conducted following scientifically acceptable standards	Y	Statistical re-analysis of 10 selected bioassays for which individual animal data are available.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Provided in the original bioassays.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Provided in the original bioassays.
Test conditions clearly and completely described	Y	Provided in the original

		bioassays.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Provided in the original assays. The maximum doses reported are 5,873 mg/kg bw/day and 4,841 mg/kg bw/day in female and male mice, respectively.
Number of animals used per dose level reported	Y	50 – 64 animals per dose group.
Method of analysis described for analysis test media	N	Should be provided in original bioassays.
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	N.A.	Statistical re-analysis of all tumor sites.
Statistical methods described	Y	Application of a multi-response permutation procedure providing valid false-positive probabilities.
Historical control data of the laboratory reported	Y	For some tumors.
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions because state-of-the-art statistical methods were employed to a selected set of cancer bioassays to demonstrate false-positive probabilities.		

Assessment and conclusion by RMS:

The publication concerns a statistical re-analysis of the available *in vivo* carcinogenicity studies on glyphosate. The individual studies are all summarized elsewhere in this RAR. The statistical analysis conducted appears to be reliable and the key elements of the material and methods and result sections is well reported. Therefore, the study is concluded to be reliable.

Although the study does not provide a lot of new information it does highlight that statistical significant effects on tumour incidences should be carefully evaluated for biological relevance as chance findings may occur with an increasing number of statistical tests. This is particular the case for glyphosate which has such a large database. For an overall evaluation of the available *in vivo* studies in the context of the classification and labelling of glyphosate we refer to Volume 1.

B.6.5.18.2. Supporting publications – Portier, 2020

Data point:	CA 5.5/027
Report author	Portier, C.J.
Report year	2020
Report title	A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies
Document No	Environ Health (2020) Vol. 19, 18. https://doi.org/10.1186/s12940-020-00574-1
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable

Previously submitted	No, submitted of the purpose of the renewal.
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: -/Reliable with restrictions Conclusion AGG: Reliable.

Abstract

Since the introduction of glyphosate-tolerant genetically-modified plants, the global use of glyphosate has increased dramatically making it the most widely used pesticide on the planet. There is considerable controversy concerning the carcinogenicity of glyphosate with scientists and regulatory authorities involved in the review of glyphosate having markedly different opinions. One key aspect of these opinions is the degree to which glyphosate causes cancer in laboratory animals after lifetime exposure. In this review, twenty-one chronic exposure animal carcinogenicity studies of glyphosate are identified from regulatory documents and reviews; 13 studies are of sufficient quality and detail to be reanalyzed in this review using trend tests, historical control tests and pooled analyses. The analyses identify 37 significant tumour findings in these studies and demonstrate consistency across studies in the same sex/species/strain for many of these tumours. Considering analyses of the individual studies, the consistency of the data across studies, the pooled analyses, the historical control data, non-neoplastic lesions, mechanistic evidence and the associated scientific literature, the tumour increases seen in this review are categorized as to the strength of the evidence that glyphosate causes these cancers. The strongest evidence shows that glyphosate causes hemangiosarcomas, kidney tumours and malignant lymphomas in male CD-1 mice, hemangiomas and malignant lymphomas in female CD-1 mice, hemangiomas in female Swiss albino mice, kidney adenomas, liver adenomas, skin keratoacanthomas and skin basal cell tumours in male Sprague-Dawley rats, adrenal cortical carcinomas in female Sprague-Dawley rats and hepatocellular adenomas and skin keratoacanthomas in male Wistar rats.

Materials and methods

Bioassay data - The animal carcinogenicity data analyzed in this publication were derived from the published literature, the EPA review, the addendum to the EFSA review prepared by the German Institute for Risk Analysis, the JMPR review, the review of the carcinogenicity of glyphosate by a panel of scientists on behalf of industry, and full laboratory reports. The 13 cancer bioassays considered acceptable for this evaluation are presented in Table B.6.5.18.2-1.

Table B.6.5.18.2-1: Characteristics of the cancer bioassays of glyphosate analyzed in this study

Table 1 Long-term chronic dietary exposure toxicity and carcinogenicity studies of glyphosate analyzed in this evaluation. Additional information on these studies is available in the Additional file 1

Study Reference	Duration (months)	Strain		Dietary exposure dose levels (mg/kg/day)	Animals per Group	Purity (%)	Comments on survival and weight
		Mouse	Rat				
A[redacted] (1983) [11]	24	CD-1		M: 0, 157, 814, 4841 F: 0, 190, 955, 5874	50	99.8	No survival differences, slight weight reduction in high dose (M)
B[redacted]t al. (1993) [12]	24	CD-1		M: 0, 98, 297, 988 F: 0, 102, 298, 1000	50	> 97.0	No survival differences, no weight differences
C[redacted] (1997) [13]	18	CD-1		M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	50	94.6–95.7	No survival differences, slight weight reduction in mid (F) & high dose (M + F)
D[redacted]l. (2009) [14]	18	CD-1		M: 0, 71.4, 234.2, 810 F: 0, 97.9, 299.5, 1081.2	51	95.7	No survival differences, no weight differences
E[redacted]1999a) [15]	18	CD-1		M: 0, 167.6, 685, 7470 F: 0, 93.2, 909, 8690	50	97.5	Reduced survival high dose (M), slight weight reduction in mid (M) & high dose (M + F). This study was only mentioned by JMPR [7] and provides limited tumor data.
F[redacted] (2001) [16]	18	S-A[a]		M: 0, 85.5, 285.2, 1077.4 F: 0, 104.5, 348.6, 1381.9	50	> 95.0	No survival differences, no weight differences
G[redacted] (1981) [17]	26		SD[b]	M: 0, 3.05, 10.3, 31.49 F: 0, 3.37, 11.22, 34.02	50	98.7	No survival differences, no weight differences
H[redacted] (1990) [18]	24		SD[b]	M: 89, 362, 940 F: 0, 113, 457, 1183	50	98.7	No survival differences, slight weight reduction in high dose (F)
I[redacted]1993) [19]	24		SD[b]	M: 0, 11, 112, 320, 1147 F: 0, 12, 109, 347, 1134	50	98.9	No survival differences, slight weight reduction in high dose (M + F)
J[redacted]1997) [20]	24		SD[b]	M: 0, 104, 354, 1127 F: 0, 115, 393, 1247	50	95.7	Reduced survival high dose (M), slight weight reduction in high dose (M + F)
K[redacted]1996) [21]	24		W[c]	M: 0, 6.3, 59.4, 595.2 F: 0, 8.6, 88.5, 886	50	96.8	No survival differences, no weight differences
L[redacted] (2001) [22]	24		W[c]	M: 0, 121, 361, 1214 F: 0, 145, 437, 1498	53	97.6	High-dose survived longer (M), reduced weight highest dose (M + F)
M[redacted]et al. (2009) [23]	24		W[c]	M: 0, 165, 838.1, 4348 F: 0, 153.2, 786.8, 4116	51	94.7–97.6	No survival differences, no weight differences

[a]Swiss Albino mouse; [b]Sprague-Dawley rat; [c]Wistar rat

For twelve of these studies full study reports were available and most of them were conducted in accordance with the appropriate regulatory guidelines. A full study report was not available for the [redacted] (1999) study but the data on kidney tumours in males and malignant lymphomas in females could be obtained from a JMPR review.

Statistical tests - Individual tumour counts for the individual studies were re-analyzed using the exact Cochran-Armitage one-sided linear trend test in proportions. Re-analyses were conducted on all primary tumours where there were at least 3 tumours in all of the animals in a sex/species/strain combination. In addition, any tumour where a significant positive trend ($p \leq 0.05$) was seen in at least one study was also evaluated in all studies of the same sex/species/strain, regardless of the number of animals with the tumour. When adenomas and carcinomas were observed in the same tissue, a combined analysis of adenomas and carcinomas was also conducted. Pairwise comparisons between individual exposed groups and the control group are conducted using Fisher's exact test. To evaluate the consistency of a tumour finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. A common positive trend is seen in the pooled analysis when the null hypothesis that the slope is 0 is rejected (statistical p-value ≤ 0.05 using a likelihood-ratio test) in favor of the alternative that the slope is greater than 0. Heterogeneity is seen in the pooled analysis when the null hypothesis that the slopes are equal is rejected (statistical p-value ≤ 0.05 using a likelihood-ratio test) in favor of the alternative that at least one of the slopes is different. For CD-1 mice analyses were conducted separately for 18 month and 24 month studies and then a combined analysis was performed. Similar grouped analyses were conducted for SD rats with one study of 26 months and 3 studies of 24 months. Only the combined analysis over all study durations is provided. The same methods of analysis were used to evaluate the incidence of non-neoplastic toxicity in tissues where tumours were observed. In cases of rare tumours where the increase in incidence didn't reach statistical significance the test proposed by Tarone (1982) was applied using an appropriate historical control group. All analyses were done using MATLAB, version R2017b.

Results

The purpose of this analysis is to understand the potential of glyphosate to produce tumours across all studies and not one study at a time. Thus, rather than presenting the results of each study separately, this review focuses on the tumours that are seen as positive in any one study and compares the findings across all studies of the same tumour in the same sex/species/strain combination.

Re-analysis of the data from CD-1 mice - From Table B.6.5.18.2-2 it can be derived that a significant ($p \leq 0.05$) positive trend was found in males for kidney adenomas and kidney adenomas/carcinomas in the [redacted] study, malignant lymphomas in the [redacted] and the [redacted] studies, hemangiosarcomas in the [redacted] study, and alveolar-bronchiolar carcinomas in the [redacted] study. When Tarone's test was applied using historical control data then a significant increase was found for kidney adenomas in the [redacted] study, kidney carcinomas in the [redacted]n study, kidney adenomas/carcinomas in the [redacted] and the [redacted] study, and hemagiosarcomas in the [redacted] study. In females a significant ($p \leq 0.05$) positive trend was found for hemangiomas, harderian gland adenomas and adenomas/carcinomas in the [redacted] study, alveolar-bronchiolar adenomas/carcinomas in the [redacted] study, and malignant lymphomas in the [redacted] study. A significant common trend was found for kidney adenomas, carcinomas and adenomas/carcinomas, hemangiosarcomas in males and hemangiomas and malignant lymphomas in females.

Table B.6.5.18.2-2: P-values for the exact Cochran-Armitage trend test and pooled logistic regression analysis for tumours with at least one significant trend test ($p \leq 0.05$) or Fisher's exact test ($p \leq 0.05$) in male and female CD-1 mice.

Tumor	Individual study *p*-values for trend[a]					Common Trend	Heterogeneity Test
Males	A	B	C	D	E		
Kidney Adenomas	0.442 (0.138)[d]	0.938	0.062 (**0.009**)[d]	---[b]	**0.019**	**0.006**	0.268
Kidney Carcinomas	0.063 (**< 0.001**)[d]	0.938	---[b]	---[b]	0.250	**0.031**	0.546
Kidney Adenomas and Carcinomas	0.065 (**0.008**)[d]	0.981	0.062 (**0.009**)[d]	---[b]	**0.005**	**< 0.001**	0.106
Malignant Lymphomas	0.754	0.087	**0.016**	**0.007**	ND[c]	0.093	0.007
Hemangiosarcomas	0.505	**0.004**	0.062 (**0.005**)[d]	---[b]	ND[c]	**0.033**	0.007
Alveolar-Bronchiolar Adenomas	0.294	0.231	0.513	0.924	ND[c]	0.384	0.409
Alveolar-Bronchiolar Carcinomas	0.918	0.456	0.148	**0.028**	ND[c]	0.407	0.083
Alveolar-Bronchiolar Adenomas and Carcinomas	0.576	0.231	0.294	0.336	ND[c]	0.346	0.826
Females	A	B	C	D	E		
Hemangiomas	0.631	---[b]	**0.002**	0.438	ND[c]	**0.031**	0.155
Harderian Gland Adenomas	0.877	ND[c]	**0.040**	0.155	ND[c]	0.155	0.052
Harderian Gland Carcinomas	---[b]	ND[c]	---[b]	1.000	ND[c]	0.500	1.00
Harderian Gland Adenomas and Carcinomas	0.877	ND[c]	**0.040**	0.372	ND[c]	0.184	0.110
Alveolar-Bronchiolar Adenomas	0.999	0.144	0.800	0.656	ND[c]	0.996	0.211
Alveolar-Bronchiolar Carcinomas	0.183	0.110	0.623	0.601	ND[c]	0.268	0.544
Alveolar-Bronchiolar Adenomas and Carcinomas	0.985	**0.048**	0.842	0.688	ND[c]	0.982	0.241
Malignant Lymphomas	0.070[e]	0.484	0.294	0.353	**0.050**	**0.012**	0.995

[a] – Study A is [redacted] [11] (Additional file 2: Table S1), Study B is [redacted] et al. [12] (Additional file 2: Table S2), Study C is [redacted] [13] (Additional file 2: Table S3), Study D is [redacted] 14] (Additional file 2: Table S4), Study E is [redacted] 15] (Additional file 2: Table S5); [b] – three dashes "---" indicates all tumor counts are zero; [c] – ND indicates there is no data available for this tumor in this study; [d] – using historical control data (see text for details) and Tarone's test; [e] – Spleen composite lymphosarcomas (malignant lymphomas) are also significantly increased in female mice in this study (see Additional file 2: Table S1)

Re-analysis of the data from Swiss albino mice - The single study with Swiss albino mice ([redacted] 2001) shows a significant increase in hemangiomas in female mice ($p = 0.004$).

Re-analysis of the data from SD rats - From Table B.6.5.18.2-3 it can be derived that a significant ($p \leq 0.05$) positive trend was found in males for testicular interstitial cell tumours in the [redacted] study, hepatocellular adenomas and adenomas/carcinomas in the [redacted] study, kidney adenomas in the [redacted] study, skin keratoacanthomas in the [redacted], [redacted] studies, and skin basal cell tumours in the [redacted] study. In females a significant ($p \leq 0.05$) positive trend was found for thyroid C-cell adenomas in the [redacted] study, C-cell carcinomas in the [redacted] study, and adrenal cortical carcinoma in the [redacted] study. When Tarone's test was applied using historical control data then a significant increase was found for pancreatic islet cell adenomas in males in the [redacted] study, and thyroid C-cell adenomas/carcinomas in females in the [redacted] study. A significant common trend was found for hepatocellular adenomas, kidney adenomas, skin keratoacanthomas and skin basal cell tumours in males. In females the common trend was statistically significant for adrenal cortical carcinoma.

Table B.6.5.18.2-3: P-values for the exact Cochran-Armitage trend test and pooled logistic regression analysis for tumours with at least one significant trend test or Fisher's exact test ($p \leq 0.05$) in male and female Sprague-Dawley rats

Tumor	Individual study *p*-values for trend[a]				Common Trend	Heterogeneity Test
Males	G	H	I	J		
Testicular Interstitial Cell Tumors	**0.009**	0.296	0.580	0.594	0.461	0.105
Pancreas Islet Cell Adenomas	0.512	0.147 (**0.007**)[c]	0.974	0.859	0.849	0.143
Pancreas Islet Cell Carcinomas	0.251	1.000	–	0.500	0.731	0.166
Pancreas Islet Cell Adenomas or Carcinomas	0.316	0.206	0.974	0.844	0.875	0.185
Thyroid C-cell Adenomas	0.743	0.089	0.278	0.631	0.210	0.532
Thyroid C-cell Carcinomas	0.505	0.442	0.495	0.565	0.322	0.898
Thyroid C-cell Adenomas and Carcinomas	0.748	0.097	0.197	0.642	0.175	0.526
Thyroid Follicular-cell Adenomas	0.122	0.408	0.067	0.966	0.464	0.055
Thyroid Follicular-cell Carcinomas	---[b]	0.255	0.443	1.000	0.448	0.137
Thyroid Follicular-cell Adenoma and Carcinoma	0.122	0.232	0.099	0.986	0.446	0.031
Hepatocellular Adenomas	0.471	**0.015**	0.325	0.500	**0.029**	0.664
Hepatocellular Carcinomas	0.062	0.637	0.760	0.642	0.803	0.269
Hepatocellular Adenomas and Carcinomas	0.173	**0.050**	0.480	0.690	0.144	0.428
Kidney Adenomas	0.938	0.813	1.000	**0.004**	**0.039**	0.002
Skin Keratoacanthomas	---[b]	**0.042**	**0.047**	**0.029**	**< 0.001**	0.998
Skin Basal Cell Tumors	0.251	0.249	1.000	**0.004**	**< 0.001**	0.009
Females	G	H	I	J		
Thyroid C-cell Adenomas	0.679	**0.049**	0.207	0.912	0.287	0.150
Thyroid C-cell Carcinomas	**0.003** (**< 0.001**)[c]	0.500	---[b]	---[b]	0.385	0.041
Thyroid C-cell Adenomas and Carcinomas	0.072 (**0.037**)[c]	0.052	0.207	0.912	0.275	0.071
Adrenal Cortical Adenoma	0.851	0.603	---[b]	0.626	0.713	0.750
Adrenal Cortical Carcinoma	0.386	**0.015**	0.493	---[b]	**0.031**	0.199
Adrenal Cortical Adenoma and Carcinoma	0.801	0.090	0.493	0.626	0.195	0.520

[a] – Study G is [redacted] [17] (Additional file 2: Table S7), Study H is [redacted] [18] (Additional file 2: Table S8), Study I is [redacted] et al. [12] (Additional file 2: Table S9) and Study J is [redacted] [20] (Additional file 2: Table S10); [b] – three dashes "---" indicates all tumor counts are zero; [c] – using historical control data (see text for details) and Tarone's test

Re-analysis of the data from Wistar rats - From Table B.6.5.18.2-4 it can be derived that a significant ($p \leq 0.05$) positive trend was found in males for hepatocellular adenomas and adenomas/carcinomas in the [redacted] study, pituitary adenomas in the [redacted] study, skin keratoacanthomas in the [redacted] study, and adrenal pheochromocytomas in the [redacted] study. In females a significant ($p \leq 0.05$) positive trend was found for mammary gland adenocarcinomas and adenomas/adenocarcinomas, pituitary adenomas and adenomas/carcinomas all in the [redacted] study. A significant common trend was found for hepatocellular adenomas and adenomas/carcinomas and skin keratoacanthomas in males. No statistically significant common trend was found for the tumours in females.

Table 6.5-1: P-values for the exact Cochran-Armitage trend test and pooled logistic regression analysis for tumours with at least one significant trend test or Fisher's exact test ($p \leq 0.05$) in male and female Wistar rats

Tumor	Individual study *p*-values for trend[a]			Common Trend	Homogeneity Test
Males	K	L	M		
Hepatocellular Adenomas	0.391	**0.008**	0.418	**0.048**	0.156
Hepatocellular Carcinomas	0.418	---[b]	1.000	0.492	0.242
Hepatocellular Adenomas and Carcinomas	0.286	**0.008**	0.610	**0.029**	0.194
Pituitary Adenomas	0.376	0.277	**0.045**	0.057	0.664
Pituitary Carcinomas	0.692	---[b]	1.000	0.771	0.956
Pituitary Adenomas and Carcinomas	0.454	0.277	0.059	0.073	0.700
Skin Keratoacanthomas	---[b]	0.387	**0.030**	**0.032**	0.823
Adrenal Pheochromocytomas	**0.048**	0.721	0.306	0.273	0.210
Females	K	L	M		
Mammary Gland Adenomas	0.539	0.941	0.062	0.448	0.015
Mammary Gland Adenocarcinomas	1.000	0.271	**0.042**	0.071	0.008
Mammary Gland Adenomas and Adenocarcinomas	0.729	0.590	**0.007**	0.113	0.064
Pituitary Adenomas	0.967	0.261	**0.014**	0.105	0.023
Pituitary Carcinomas	1.000	-	0.750	0.748	0.491
Pituitary Adenomas and Carcinomas	0.976	0.261	**0.017**	0.129	0.019

[a] – Study J is [redacted]21] (Additional file 2: Table S11), Study K is [redacted]22] (Additional file 2: Table S12), and Study L is [redacted]t al. [14] (Additional file 2: Table S13); [b] – three dashes "---" indicates all tumor counts are zero

False positive errors - The evaluation of any one animal cancer study involves a large number of statistical tests that could lead to false positives. To evaluate this issue, the probability that all of the results in any sex/species/strain could be due to false positive results is calculated. Overall, a total of 496 evaluations were done for these 13 studies including the few evaluations done against historical controls. There are 41 evaluations at 37 tumour/site combinations with a trend test $p \leq 0.05$. The probability that all of these are due to false positives is 0.001. Similarly, looking at the evaluations resulting in $p \leq 0.01$, the probability that all of the findings are due to false positives is < 0.001. The strongest evidence was found for male CD-1 mice where the probability for 11 positive findings to occur at $p \leq 0.05$ and 8 positive findings at $p \leq 0.01$ are both below 0.001.

Conclusions

Oral exposure of rats and mice to glyphosate *via* the diet in 13 separate carcinogenicity studies demonstrates that glyphosate causes a variety of tumours that differ by sex, species, strain and length of exposure. To summarize the results of the strength-of-evidence analysis, each tumour is placed in any of the following categories: Clear evidence (CE) when there is a causal link between glyphosate exposure and the tumour; Some evidence (SE) when there is a causal link between glyphosate exposure and the tumour but chance, although unlikely, cannot be ruled out; Equivocal evidence (EE) when there is a causal link between glyphosate exposure and the tumour but chance is as likely an explanation for the association as is exposure to glyphosate; No evidence (NE) indicates that any causal link between glyphosate exposure and the tumour is almost certainly due to chance. The factors used to place tumours into these categories include the analyses of the individual studies, the consistency of the data across studies (pooled analyses), the analyses using historical control data, the analyses of non-neoplastic lesions, mechanistic evidence and the associated scientific literature.

The weight-of-evidence analysis conducted in this study indicates that there is clear evidence (CE) that oral exposure to glyphosate *via* the diet produces adrenal cortical carcinoma in the female SD rat, hemangioma in the female mouse (CD-1 and Swiss albino), hemangiosarcoma in the male CD-1 mouse, kidney tumours in the male CD-1 mouse and SD rat, liver adenoma in male rats (SD and Wistar), malignant lymphoma in the male and female CD-1 mouse, skin basal cell tumour in the male SD rat and skin keratoacanthoma in male rats (SD and Wistar). Some evidence (SE) for a causal relationship was found for kidney tumours in male Swiss albino mice, mammary

tumours in female Wistar rats, malignant lymphoma in male and female Swiss albino mice, pituitary adenoma in the male and the female Wistar rat, and testicular interstitial cell tumours in the male SD rat. The results of the analyses conducted in this study are supportive of the conclusions of IARC that there is sufficient evidence to consider glyphosate as a rodent carcinogen.

Assessment and conclusion by applicant:

Thirteen glyphosate cancer bioassays considered acceptable for this re-analysis were selected from the published literature, the EPA review, the review from the German Institute for Risk Analysis, the JMPR review, and full laboratory reports. For twelve of them full study reports were available. Individual tumour counts for the individual studies were re-analyzed using the exact Cochran-Armitage one-sided linear trend test. Re-analyses were conducted on all primary tumours where there were at least 3 tumours in all of the animals in a sex/species/strain combination. In addition, any tumour where a significant positive trend ($p \leq 0.05$) was found in at least one study was also evaluated in all the other studies of the same sex/species/strain combination, regardless of the number of animals with the tumour. Pairwise comparisons between individual exposed groups and the control group were conducted using Fisher's exact test. To evaluate the consistency of a tumour finding across multiple studies using the same sex-species-strain combinations, logistic regression with individual background responses and dose trends are fit to the pooled data using maximum likelihood estimation. The same methods of analysis were used to evaluate the incidence of non-neoplastic lesions in tissues where tumours were observed. In cases of rare tumours where the increase in incidence didn't reach statistical significance the test proposed by Tarone (1982) was applied using an appropriate historical control group. To summarize the results of the strength-of-evidence analysis, each tumour is placed in any of the following categories: Clear evidence (CE), some evidence (SE), equivocal evidence (EE), and no evidence (NE). The factors used to place tumours into these categories include the analyses of the individual studies, the consistency of the data across studies (pooled analyses), the analyses using historical control data, the analyses of non-neoplastic lesions, and mechanistic evidence with the associated scientific literature. The author's weight-of-evidence analysis indicates that there is clear evidence (CE) that oral exposure to glyphosate *via* the diet produces adrenal cortical carcinoma in the female SD rat, hemangioma in the female mouse (CD-1 and Swiss albino), hemangiosarcoma in the male CD-1 mouse, kidney tumours in the male CD-1 mouse and SD rat, liver adenoma in the male rat (SD and Wistar), malignant lymphoma in the male and female CD-1 mouse, skin basal cell tumour in the male SD rat and skin keratoacanthoma in male rats (SD and Wistar). Some evidence (SE) for a causal relationship was put forth for kidney tumours in the male Swiss albino mouse, mammary tumours in the female Wistar rat, malignant lymphoma in the male and female Swiss albino mouse, pituitary adenoma in the male and the female Wistar rat, and testicular interstitial cell tumours in the male SD rat.

After thorough analysis and considering all factors that are important in the interpretation of cancer studies none of the tumours identified by the author as indicating clear evidence (CE) or some evidence (SE) of carcinogenicity were found relevant for reconsideration under the necessary due diligence of the European AIR5 review of glyphosate. Most of the tumours selected by the author were previously dismissed by the EU experts as not relevant even before the last review of glyphosate in 2017, and the applicant believes that there is no solid toxicological evidence for glyphosate exposure related carcinogenicity in the mouse and the rat that warrants any science-based concerns for human health. The discussion of each of the suspect tumours is given below.

Clear evidence (CE) for carcinogenicity:

Adrenal cortical carcinoma in the female SD rat [redacted] *study):*

The tumour incidences were 0/60, 0/60, 0/60, 3/60 at 0, 113, 457, and 1183 mg/kg bw/day, respectively. This tumour has not been considered treatment related by the authors of the study. There is no dose-related increase in adrenal cortical adenoma (1/60, 3/60, 2/60, 1/60), no dose-related increase in pre-neoplastic lesions and this tumour was not found in the males of the same study or in other rat studies. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female CD-1 mice ([redacted] *study):*

In the [redacted] study hemangiomas were observed in different tissues:

- In liver with an incidence of 0/50, 0/50, 1/50, 1/50;
- In the ovary with an incidence of 0/50, 0/50, 0/50, 1/50;
- In the uterus with an incidence of 0/50, 0/50, 1/50, 2/50;
- In the spleen with an incidence of 0/50, 0/50, 1/50, 0/50;

- In the abdominal cavity with an incidence of 0/8, 0/9, 0/9, 1/9;

At 0, 153.2, 786.8, and 4116 mg/kg bw/day, respectively. Taken together as systemic tumours a significant positive trend is obtained. However, hemangiomas have also been observed in males (liver and testes) but without any dose-response relationship and the highest incidence found was in the control group (1/50). These tumours have not been confirmed in the other carcinogenicity studies in the CD-1 mouse. Moreover, the dose level (4116 mg/kg bw/day) at which the incidence was statistically significantly increased when compared against the control, is more than 4-fold the limit dose for the testing of carcinogens in rodent species. If that dose is ignored there is no significative positive trend. Therefore, this tumour has been considered not relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

Hemangioma in female Swiss albino mice ([redacted] study):
In the re-analysis of the tumour data of the [redacted] study by [redacted] (report submitted in 2017) no statistically significant trend was found for systemic neoplasms using the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto- and endoparasites in a large number of animals. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Hemangiosarcoma in the male CD-1 mouse ([redacted] study):
The tumour incidences were 0/50, 0/50, 0/50, 4/50 (3 in liver and 1 in prostate) at 0, 98, 297, and 988 mg/kg bw/day, respectively. The incidence at the highest dose (8 %) was still within the historical control range of the test laboratory (0-8 %, 300 male mice in 6 studies up to 1993). This tumour was not confirmed in other mouse studies of which one ([redacted] study) with a dose level nearly 5-fold that of the [redacted] study (4841 mg/kg bw/day). Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male CD-1 mouse ([redacted] study, as reported by JMPR, 2016):
Renal cell adenoma (3/50) and renal cell carcinoma (1/50) were observed in males at 7470 mg/kg bw/day, but, according to the authors, there was no statistically significant difference with the control group. It is of note that the high dose considered in this study for males is extraordinarily high, more than 7-fold the limit dose for the testing of carcinogens in rodent species. If this dose is ignored there is no significant positive trend. These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel of the Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. After re-examination, the incidences for renal cell adenoma were 1/50, 1/50, and 1/50 at 167.6, 685, and 7470 mg/kg bw/day, showing no dose-response relationship. The incidence for renal cell carcinoma was confirmed to be 1/50 at 7470 mg/kg bw/day. The historical control data for the [redacted] study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13 %) in males and 0/725 (0 %) in females and for renal cell adenoma were 3/564 (0.53 %) in males and 0/564 (0 %) in females. The re-examination report also provides reference data of 0-1.8 % in males and 0 % for all doses in females for renal cell carcinoma, and 0-1.8 % in males and females for renal cell adenoma. The results of the re-examination revealed also that the tubular epithelial cell hypertrophy was localized with an incidence in each treatment group that did not significantly differ from that in the control group. There was no association between the tubular epithelial cell hypertrophy and the development of renal tumours. The renal cell tumours observed in this study are thus not relevant for the human risk assessment of glyphosate because (1) the incidence of renal tumours in males at 7470 mg/kg bw/day did not significantly differ from that in the control group upon re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the high dose considered in this study for males is more than 7-fold the limit dose for the testing of carcinogens in rodent species. Therefore, this tumour has been considered not relevant for the assessment of glyphosate.

Kidney tumours in the male SD rat ([redacted] study):
The incidences of kidney adenoma were 0/76, 0/75, 0/80, 4/78 at 0, 104, 354, and 1127 mg/kg bw/day. An increasing trend in the incidence of adenomas in the kidney was observed in males of the high dose group (animal 193: killed in extremis at week 92, animal 167: found dead at week 94, animal 159: found dead at week 101, and animal 169: killed by design after 104 weeks of treatment) and this incidence was greater than the historical control range referred to in the study report (0-2.9 %). However, according to the authors of this study, the increase observed was not statistically significant. No kidney tumours were found in the females and nearly all male rats at all dose levels suffered from chronic nephropathy (62/76, 63/75, 56/80, 67/78). This tumour in this study was not considered relevant for the risk assessment of glyphosate and was not discussed

further in the previous EU review of glyphosate.

Hepatocellular adenomas in the male SD rat ([redacted] study):
The tumour incidences for adenomas were 3/60, 2/60, 3/60, 8/60 and of carcinomas were 3/60, 2/60, 1/60, 2/60 at 0, 89, 362, and 940 mg/kg bw/day, respectively. The incidence of adenomas at the high dose (13.3 %) is still within the historical control range of the test laboratory (1.4-18.3 %). Foci of cellular alteration were observed at all dose levels without any dose-response relationship and there were no signs of hepatocellular hypertrophy, a prerequisite for hepatocellular carcinogenesis. Beside the [redacted] study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Hepatocellular adenomas in the male Wistar rat ([redacted] study):
The tumour incidences were 0/64, 2/64, 0/64, 5/64 at 0, 121, 361, 1214 mg/kg bw/day, respectively. The positive trend is significant and the incidence at the high dose is significantly different from the control. However, the incidence at the high dose (7.8 %) is still within the historical control range of the test laboratory (0-11.5 %, 26 studies in 1984-2003). There were no histopathological signs of liver enzyme induction or pre-neoplastic lesions. The high dose animals in this study survived longer when compared to the other groups. This may also influence the spontaneous tumour rate. Beside the [redacted] study no increase in hepatocellular adenomas was noted in the other rat studies. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male CD-1 mouse ([redacted] study):
The tumour incidences were 2/50, 2/50, 0/50, 6/50 at 0, 165, 838, 4348 mg/kg bw/day, respectively. The positive trend is significant but the incidence at the high dose is not significantly different from the control. Moreover, the incidence at the high dose (12 %) is still within the historical control range of the test laboratory (3.6-19.2 %, 458 male mice in 12 studies in 1993-1998). The trend has been found significantly positive because of the elevated incidence at a dose level that is over 4-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is not positive. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the female CD-1 mouse ([redacted] study):
The tumour incidences were 3/50, 1/50, 4/50, 6/50 at 0, 93.2, 909, and 8690 mg/kg bw/day, respectively. The increased incidence of lymphoma at the high dose was statistically significant in the trend test but not in a pairwise comparison. The trend has been found significantly positive because of the elevated incidence at an extraordinarily high dose level, more than 8-fold the limit dose for carcinogenicity studies in rodents. If this dose is ignored the trend is no longer significant. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Skin basal cell tumour in the male SD rat ([redacted] study):
The tumour incidences were 0/78, 0/75, 0/80, 3/78 for adenoma and 0/78, 0/75, 0/80, 1/78 for carcinoma at 0, 104, 354, and 1127 mg/kg bw/day. No increased incidence of this tumour was observed in the females or other rat studies and may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for the adenomas, the increase in incidence at the high dose level was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat ([redacted] study):
The tumour incidences were 1/60, 3/60, 4/60, 5/60 at 0, 89, 362, and 940 mg/kg bw/day. Although there is a significant positive trend the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate by the authors of this study. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat ([redacted] study):
The combined incidences of intracutaneous cornifying epithelioma (keratoacanthoma) were 1/50, 2/25, 0/19, 0/21, 5/50 at 0, 11, 112, 320, and 1147 mg/kg bw/day. Although the trend was significant, the incidence at the high dose was not statistically significantly different from the control and considered not related to treatment by the authors of this study. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms

in male Sprague Dawley rats. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male SD rat ([redacted] study):
The incidences of the tumour were 4/76, 3/75, 0/80, 7/78 at 0, 104, 354, and 1127 mg/kg bw/day. The increased incidence of this skin tumour at the high dose may be associated with other skin lesions (follicular hyperkeratosis and/or folliculitis/follicular abscess) observed in this study. Although there is a significant positive trend for this tumour, the increase in incidence at the high dose level was not statistically significantly different from the control. Skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats and considered by the authors of this study not relevant for the risk assessment of glyphosate. This tumour was not discussed further in the previous EU review of glyphosate.

Skin keratoacanthoma in the male Wistar rat ([redacted] study):
There were no treatment-related conditions seen in the skin or in subcutaneous tissues, but several spontaneous lesions were observed. Epidermal ulceration and scab formation, inflammatory lesions, abscess formation, focal acanthosis, focal mineralisation, focal dermal thickening, and focal necrosis were seen, occasionally or rarely and without significance. This tumour was not discussed further in the previous EU review of glyphosate.

Some evidence for carcinogenicity (SE)
Kidney tumours in the male Swiss albino mouse ([redacted] study):
In the re-analysis of the tumour data of the [redacted] study by [redacted] (submitted in 2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Mammary tumour in the female Wistar rat ([redacted] study):
At interim and terminal sacrifice combined mammary neoplasia was seen in 6 female mice. There were no mammary neoplasms in the control group but carcinomas were seen with incidences of 2/51, 3/51, and 1/51 at 153.2, 786.8, and 4116 mg/kg bw/day, respectively. All neoplasms were adenocarcinomas with the exception of one adenosquamous carcinoma seen in a low dose group animal. No increase in the incidence of these tumours was reported in the females of other rat studies. The authors concluded that there was no effect of treatment upon the incidence of mammary neoplasia in this study. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Malignant lymphoma in the male Swiss albino mouse ([redacted] study):
In the re-analysis of the tumour data of the [redacted] study by [redacted] (2017) no statistically significant trend was found for systemic neoplasms in the Peto test. When analyzed using the Fischer's exact test no statistically significant increase in incidence was found in pair-wise comparisons of all dose groups with the control group. It is important to emphasize that this study was compromised by non-identified ecto-and endoparasites in a large number of animals. Therefore, this tumour was not considered as relevant for the assessment of glyphosate.

Pituitary adenomas in the male and the female Wistar rat ([redacted] study):
Pituitary adenomas were only seen in female mice with incidences of 0/51, 1/51, 0/51, 2/52 at 0, 104.5, 348.6, and 1381.9 mg/kg bw/day. The group distribution was unrelated to treatment. Therefore, this tumour was not considered relevant for the assessment of glyphosate.

Testicular interstitial cell tumour in the male SD rat ([redacted] study):
The incidences of this tumour were 0/50, 3/50, 1/50, 6/50 at 0, 3.05, 10.30, and 31.49 mg/kg bw/day, respectively. The positive trend is statistically significant and the incidence at the high dose level (12 %) is statistically significantly different from the control and greater that the historical control rate of the test laboratory (3.4-6.6 %). However, there was no dose-response relationship for interstitial cell hyperplasia (1/50, 1/50, 1/50, 0/50). Since the dose range considered in this study (0-31.5 mg/kg bw/day) is approximately at least 30-fold lower than that of all the other studies in rats where no increase of such tumours was found this finding should be considered as spontaneous in nature. Therefore, this tumour was not considered relevant for the risk assessment of glyphosate and was not discussed further in the previous EU review of glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions

because some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been previously dismissed in the EU regulatory process.

Reliability criteria for *in vivo* toxicology studies

Publication: Portier, 2020.	Criteria met? Y/N/?	Comments
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N.A.	
Study performed according to GLP	N. A.	Most of the study reports analysed were GLP compliant.
Study completely described and conducted following scientifically acceptable standards	Y	Re-analysis of the tumour data of 13 selected glyphosate cancer bioassays.
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of glyphosate used in every cancer bioassay mentioned.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	
Test conditions clearly and completely described	N.A.	Described in the test reports of 12 of the selected studies. The data from one study were derived from a JMPR review.
Route and mode of administration described	Y	Oral *via* the diet.
Dose levels reported	Y	Dose range from 71.4 to 8690 mg/kg bw in the mouse and from 3.05 to 4348 mg/kg bw in the rat.
Number of animals used per dose level reported	Y	About 50 per dose group.
Method of analysis described for analysis test media	N.A.	Described in the original test reports.
Validation of the analytical method	N.A.	
Analytical verifications of test media	N.A.	
Complete reporting of effects observed	Y	
Statistical methods described	Y	All statistical methods used in the re-analysis of the tumour data were reported, however sometimes not in sufficient detail.
Historical control data of the laboratory reported	N.A.	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because some of the statistical methods employed were not described in sufficient detail. Besides, the results of this study are not in agreement with the findings of Crump *et al*. 2020 in relation to the estimation of false		

positives and the overall evaluation of the significance of the tumours by the EU regulatory authorities. All the tumours that were identified by the author as providing clear evidence for the carcinogenicity of glyphosate have been dismissed in the EU regulatory process.

Assessment and conclusion by RMS:

The publication concerns a statistical re-analysis of available *in vivo* carcinogenicity studies on glyphosate. The individual studies are all summarized elsewhere in this RAR with the exception of the study by [redacted] *et al.* for which only a brief summary in the JMPR evaluation is available.

Overall, it is concluded that all key elements of the materials and methods and results section are adequately described and therefore the study is concluded to be reliable.

All observed findings in the context of the classification of glyphosate are discussed in Volume 1.

B.6.5.18.3. Supporting publications – Wozniak, 2020

Data point:	CA 5.5/028
Report author	Wozniak, E. *et al.*
Report year	2020
Report title	Glyphosate affects methylation in the promoter regions of selected tumor suppressors as well as expression of major cell cycle and apoptosis drivers in PBMCs (*in vitro* study)
Document No	doi.org/10.1016/j.tiv.2019.104736 E-ISSN: 1879-3177
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Conclusion GRG : Yes/Reliable without restrictions Conclusion AGG : Reliable without restrictions (Klimisch score 1)

Full summary of the study according to OECD format

The effect of glyphosate on selected epigenetic parameters and major cell cycle drivers in human peripheral blood mononuclear cells (PBMCs) was determined. The cells were incubated with glyphosate at 0.5, 10 and 100 μM. The analysis included: global DNA methylation, methylation in the promoter regions of tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1) by the Real-Time PCR and the expression profile of the indicated genes by Real-Time PCR. The obtained results have revealed significant reduction of global DNA methylation level in PBMCs exposed to glyphosate. Tested compound changed methylation pattern of the P21 and TP53 suppressor gene promoters, but in case of other analysed genes: P16, BCL2 and CCND1 we did not identify any statistically significant changes. Gene profiling showed that glyphosate changed the expression of genes involved in the regulation of cell cycle and apoptosis. Glyphosate decreased expression of P16 and TP53 as well as an increase in the expression of BCl2, CCND1 and P21. Summing up, our results have shown a potential disturbance in methylation processes and gene expression in human PBMCs exposed to glyphosate, but the observed changes do not prejudge about the final metabolic effects, which are depended on many

other factors.

Materials and methods

Chemicals; N-(phosphonomethyl)glycine (glyphosate) (purity 95 %), fetal bovine serum (FBS), penicillin – streptomycin, TRIzol™ and using primers were bought from Sigma-Aldrich, (USA). RPMI 1640 medium with L-glutamine and lymphocyte separation medium (LSM) (1.077 g/cm^3) were purchased in Cytogen (Germany). Invisorb Spin Tissue Mini Kit was bought in Stratec (Germany). Methylated DNA Quantification Kit was bought in Abcam (United Kingdom). Transcriptor First Strand cDNA Synthesis Kit and FastStart Essential DNA Green Master was purchased from Roche (Basel, Switzerland). EZ DNA Methylation™ Kit was bought from Zymo Research (USA). Methyl Primer Express®, v.1.0 was obtained from Life Technologies. TRIzol™ Reagent was purchased from Thermo Fischer Scientific, Waltham, MA, USA. Other chemicals were from Roth (Germany) and POCh (Poland) and were of analytical grade.

Cells isolation; PBMCs were isolated from leucocyte-buffy coat obtained from blood purchased in Blood Bank in Lodz, Poland. Blood was obtained from four healthy volunteers (aged 18–55), who showed no signs of infection disease symptoms at the time the blood samples were collected. The investigation was approved by the Bioethics Committee of the University of Lodz No. 1/KBBN-UŁ/II/2017. Cells isolation was determined. The final PBMCs density used in the experiments (after addition of glyphosate) was 1×10^6 cells/mL.

Cells treatment; Glyphosate was dissolved in PBS, pH 7.4. The concentrations of glyphosate were from 0.5 to 100 μM (0.085–17 mg/L). In a previous study, no changes in cell viability after treatment of PBMCs with glyphosate were observed at the above mentioned concentrations. The cells were incubated with investigated xenobiotic for 24 h in four independent experiments (four blood donors). During incubation, the cells were resuspended in RPMI supplemented with 10 % FBS and penicillin/streptomycin solution (50 U/mL and 50 μg/mL, respectively) at 37 °C, 5 % CO_2. After incubation, the cells were centrifuged, glyphosate was discarded, and the cells were resuspended in RPMI medium.

Methylation levels

Global DNA methylation; Genomic DNA from human PBMCs was isolated using Invisorb Spin Tissue Mini Kit (Stratec Molecular GmbH, Berlin, Germany). Global DNA methylation was determined by colorimetric measurement of 5-methylcytosine in DNA using Methylated DNA Quantification Kit (Abcam). For global DNA methylation analysis, 100 ng of genomic DNA was used, following the protocol provided by the manufacturer. Methylation levels were calculated relatively to the methylated control DNA (included in the kit) and expressed as a percentage of total methylated DNA using the following formula:

$$5-\mathrm{mC}\% = \frac{(\text{Sample OD} - \text{Negative Control OD}) \div S)}{\text{Positive Control OD} - \text{Negative Control OD}) \times 2 \div P} \times 100\%$$

where:

S – the amount of input sample DNA in ng; P – the amount of input positive control in ng; 2 – a factor to normalize 5-mC in the positive control to 100 %, as the positive control contains only 50 % of 5-mC.

Methylation at promoter regions of tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1); Chemical modification of 500 ng of genomic DNA was analysed using EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA, US), according to manufacturer's instruction. For methylation analysis, methylation-specific real-time PCR assay (MSP-PCR) was conducted with FastStart SYBR Green Master (Roche, Basel Switzerland). Bioinformatic analysis of the potential

methylation sites within the promoter regions of the proto-oncogens BCL2 and CCND1 as well as methylated and unmethylated primers were designed by utilizing of Methyl Primer Express®, v.1.0 (Life Technologies, Carlsbad, CA, US). The DNA sequences around the transcription start sites (from -1000 to 300 bp) of both genes, were obtained from the DBTSS. The primer list is presented in Supplementary Table 1. All samples were amplified in duplicate and in the presence of positive control (CpG methylated Jurkat genomic DNA, fully methylated), negative control (5-Azadc-treated Jurkat genomic DNA) (NEB, Ipswich, MA, US) and blank control (water). The methylation status of a particular gene is expressed as methylation index (MI):

$$\Delta Ct = CtU - CtM$$

$$MI = \frac{1}{1 + 2^{-\Delta Ct}} \times 100\%$$

where: MI - methylation index [%]; Ct values of the methylated gene (M) were compared with the Ct values of the unmethylated gene (U).

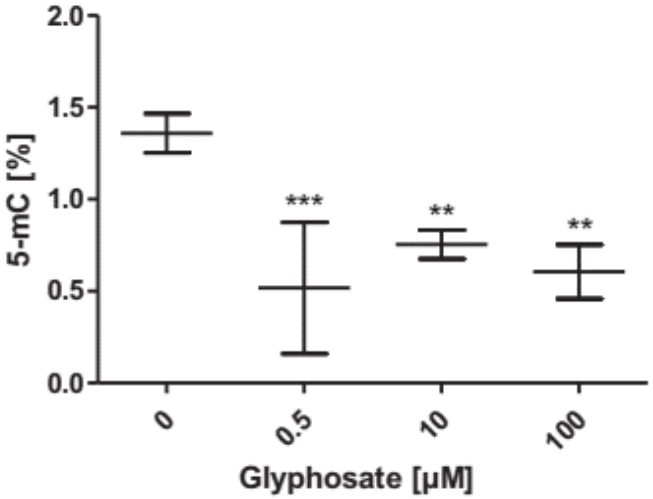
Gene expression: RNA was extracted with TRIzol™ Reagent (Thermo Fischer Scientific, Waltham, MA, USA). RNA samples with a 260/280 nm ratio in the range of 1.8–2.0 were used for further analysis. cDNA synthesis was performed using Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel Switzerland). The cDNA was quantified by real-time PCR using FastStart SYBR Green Master (Roche, Basel Switzerland). Gene expression was normalized to the mean expression of all three housekeeping genes (GAPDH, RPL13, RPLP0) and was presented as a relative gene expression. The 2ΔCt (Ctgene–Ct mean from GAPDH, RPL13, RPLP0) method was used to calculate the expression levels of studied genes. The $2^{-\Delta Ct} \times 100$ values were re-calculated into relative copy number values. Primers were designed using Primer-BLAST NCBI – NIH website and DNA sequence of selected genes were obtained using the NCBI Reference Sequences database.

Statistical analysis; The statistical analysis was performed with STATISTICA 13.1 data analysis software (2000 Stat-Soft, Inc., Tulsa, USA). Statistical analysis was conducted using the one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons procedure. The difference was considered to be significant for $P < .05$. The individual analysis was performed on blood from four donors, while each experiment (conducted for blood from one donor) was repeated twice or three times depending on the method.

Results

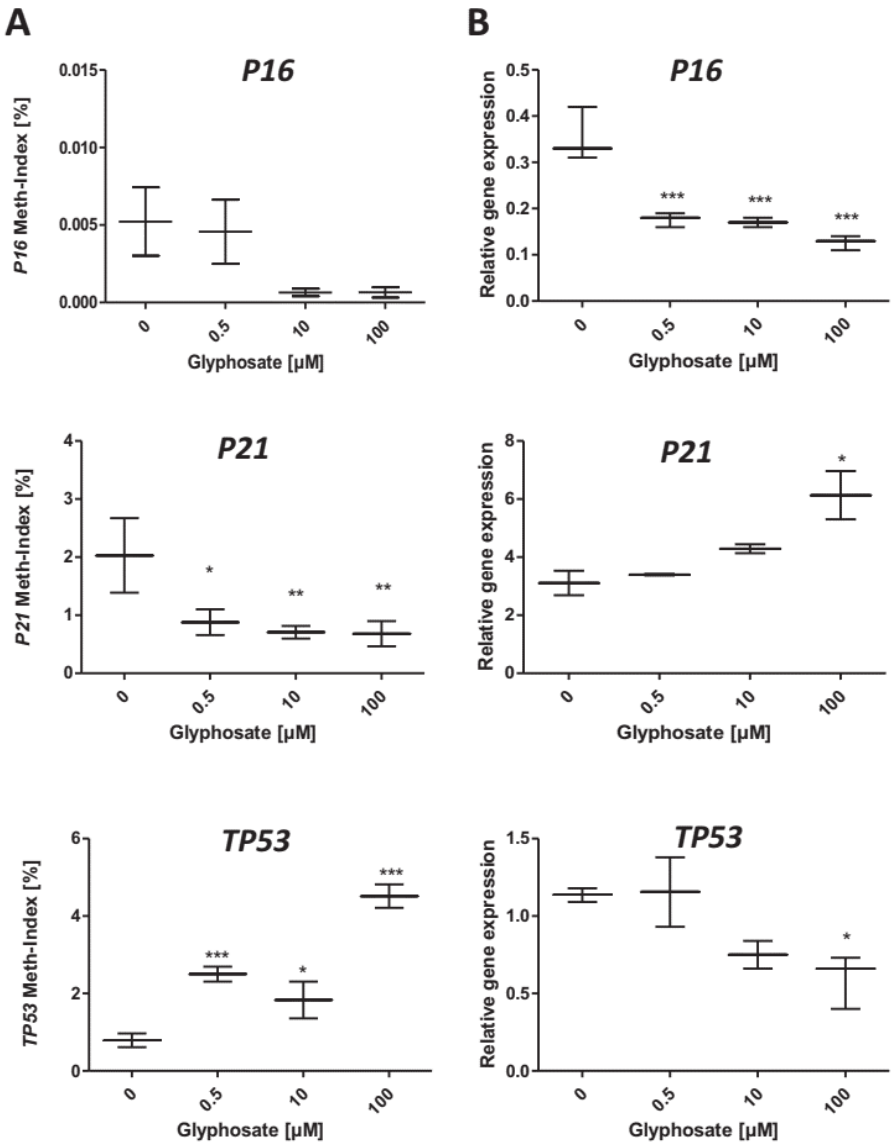
Analysis of global DNA methylation level; Statistically significant changes in 5-mC level were observed in PBMCs treated with glyphosate (see figure below). As compared to control cells, the level of global DNA methylation was significantly decreased after glyphosate treatment at all concentrations tested: 0.5 μM, 10 μM and 100 μM.

Figure B.6.5.18.3-1: 5-methylcytosine (%) in human PBMCs incubated with glyphosate (0.5-100 µM) for 24 h. Mean ± SD was calculated for four individual experiments. Statistically different from control at **p < 0.01; *** < 0.0001. Statistical analysis was conducted using one-way ANOVA and a posteriori Tukey test.



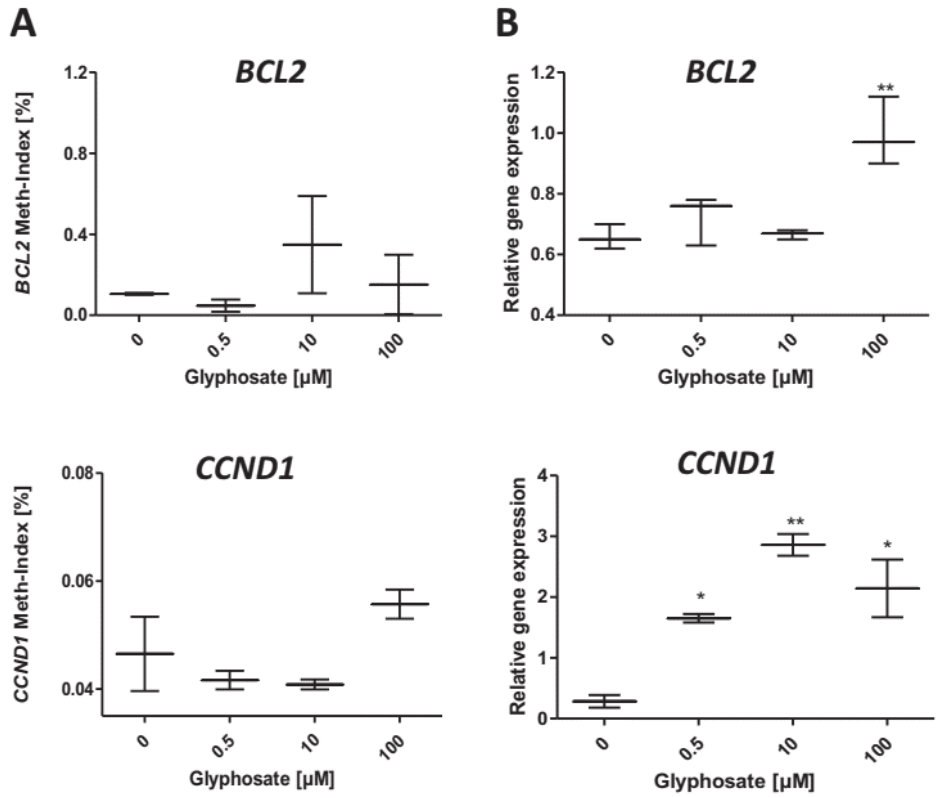
Analysis of methylation at promoter regions of the selected tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1); Statistically significant ($p < 0.05$, one-way ANOVA) decrease of methylation within P21 gene promoter was found in PMBCs treated with glyphosate from its lowest concentration of 0.5 µM (Figure B.6.5.18.3-2A). The opposite response to the investigated xenobiotic (0.5 µM) such as hypermethylation of gene promoter, was observed on TP53 tumor suppressor gene (Figure B.6.5.18.3-2A). In the case of other analyzed genes: P16, BCL2 and CCND1 we did not determine statistically significant changes in gene promoter methylation level (Figure B.6.5.18.3-2A and Figure B.6.5.18.3-3A).

Figure B.6.5.18.3-2: Methylation (A) and expression (B) of suppressor genes (P16, P21 and TP53) in human PBMCs incubated with and glyphosate (0.5-100 µM) for 24 h. *Mean ± SD was calculated for four individual experiments. Statistically different from control at *p < 0.05, **p < 0.01; *** < 0.0001. Statistical analysis was conducted using one-way ANOVA and a posteriori Tukey test.*



Analysis of gene expression of the selected tumor suppressor genes (P16, P21, TP53) and proto-oncogenes (BCL2, CCND1); The analysis of methylation of the selected gene promoters using Real-Time PCR let us assess their expression at the transcript level. A statistically significant decrease of P16 expression in PBMCs treated with all concentrations of glyphosate were identified (Figure B.6.5.18.3-2B). Glyphosate induced significant decrease in TP53 expression only at the highest concentration of 100 µM ($p < 0.05$, one-way ANOVA). Expression of other cell cycle drivers like P21 demonstrated a significant increase only at the highest concentration of glyphosate ($p < 0.05$, one-way ANOVA; Figure B.6.5.18.3-2B), which was also effective in increasing of BCL2 expression (Figure B.6.5.18.3-3B). Expression of the cyclin was significantly increased by two tested concentrations (10 µM and 100 µM) of glyphosate ($p < .05$, oneway ANOVA) (Figure B.6.5.18.3-3B).

Figure B.6.5.18.3-3: Methylation (A) and expression (B) of proto-oncogenes (BCL2 and CCND1) in human PBMCs incubated with and glyphosate (0.5-100 µM) for 24 h. *Mean ± SD was calculated for four individual experiments. Statistically different from control at *p < 0.05, **p < 0.01. Statistical analysis was conducted using one-way ANOVA and a posteriori Tukey test.*



Discussion

The knowledge that pollutants may influence the epigenome raises grave concerns concerning their long-term effects on chronic diseases development. A study (Duforestel et al. 2019) showed that glyphosate can predispose breast cells to tumorigenesis *via* epigenetic reprogramming, which occur through TET3-mediated global and local DNA hypomethylation. Just recently, changes in the DNA methylation machinery due to glyphosate treatment were also identified in the fish model of Japanese medaka (Oryzias latipes), where upregulation of DNMT1, DNMT3a, Tet1 and Tet3 gene expression was shown (Smith et al, 2019). The current results have shown that treatment of PBMCs with glyphosate changed global DNA methylation profile. Decreased 5-mC level in PBMCs treated with low concentration of glyphosate that is comparable to that determined in human body after environmental exposure (0.5 µM) were found. Moreover, the obtained results are in agreement with previous findings, showing that glyphosate at high concentrations (determined in blood during glyphosate acute poisoning at 250–500 µM), reduces total DNA methylation in PBMCs. Recently, a study (Duforestel et al. 2019) observed that glyphosate causes global DNA hypomethylation in non-neoplastic mammary epithelial MCF10A cells and triggers tumorigenesis in a "two-hit oncogenic model". Also a specific DNA hypomethylation signature of genes (i.e. local DNA hypomethylation), which was linked to the TET3 pathway that potentially can be used as an epimark to glyphosate exposure, was identified. Similar findings i.e. global hypomethylation of DNA were found in blood cells of tobacco farmers (n = 40) using pesticides and other xenobiotics during plant cultivation (Kahl et al. 2018). However, literature data regarding potential epigenetic effects exerted by herbicides became contradictory. Recent epidemiological studies point to an increase of DNA methylation level under the combined effects of various pesticides, including glyphosate (Benedetti et al. 2018). It was found that long-term exposure of farmers (n = 137), employed at growing soybeans cultivation, to complex mixtures of pesticides, resulted in DNA hypermethylation and micronuclei formation in their blood cells probably due to impairment of DNA repair mechanisms. Nevertheless, the farmers were exposed to the mixture of different pesticides and heavy metals that are known to induce DNA hypermethylation and to disturb the cell cycle progression (Chanda et al. 2006). These findings were confirmed in a study on children

living in an industrial areas. In blood samples of young subjects exposed to increased heavy metals and polycyclic aromatic hydrocarbons concentrations, DNA hypermethylation was observed (Alvarado-Cruz et al. 2017). Detailed analysis of selected genes promoters involved in cellular metabolism regulation including cell cycle, i.e. TP53, P21, P16, CCND1 or BCL2, revealed significant changes in their methylation profiles. Beside global DNA hypomethylation we have found an increased methylation within the CpG islands of the TP53 gene promoter in PBMCs treated with glyphosate in all range of concentrations. The lowest concentration (0.5 μM) of glyphosate induced 5-mC methylation of the TP53 gene promoter. This result is in agreement with previous studies, where observed statistically significant increase of CpG islands methylation within the TP53 gene promoter after treatment of PBMCs with 250 μM and 500 μM glyphosate were identified. TP53 is known to be genome guard and tumor suppressor gene. It has been shown that hypermethylation of CpG islands within its promoter results in the silencing of gene transcription, but it is not clear-cut relationship in this case (Song et al. 2015). Glyphosate reduced TP53 expression only from the concentration of 100 μM. Changes in TP53 expression and identified functions of p53 protein in the regulation of cell cycle directed us towards the findings of Marc et al. 2002, who have shown that only Roundup but not glyphosate changed cell cycle of sea urchin embryonic cells due to delayed activation of the CDK1/cyclin B complex, acting downstream from p53. The effect of glyphosate on cell cycle was also excluded in the experiments on human lymphocytes; however the tested concentrations range of this compound was between 0.0125 and 0.5 μg/mL (0.07–2.9 μM) (Santovito et al. 2018), while the current study showed that glyphosate at 100 μM caused downregulation of TP53 expression in PBMCs. Beside hypermethylation of TP53 gene promoter and down regulation of its transcript level in PMBCs treated with glyphosate (100 μM), hypomethylation within P21 gene promoter was detected for all glyphosate concentrations. Changes in methylation of the P21 gene promoter were correlated with increased expression of the cell cycle regulator at 100 μM of glyphosate, that would suggest inhibition of PBMCs cycling in G0/G1 phase. Further analysis of molecular drivers of the cell cycle revealed a decreased expression of the P16 gene encoding the cell cycle inhibitor in glyphosate-treated cells, staring from its lowest concentration comparable to that occurring during environmental exposure. However, the analysis carried out did not show any changes in the methylation level within the P16 gene promoter region in PBMCs due to glyphosate treatment. These results correlate with previous findings, in which higher concentrations of glyphosate (250 μM and 500 μM) also did not cause any changes in methylation of P16 gene promoter. The protein product of P16 gene, p16, is an inhibitor of cyclin D. Literature data suggests that low expression of P16 may result in overexpression of cyclin D1 (CCND1), activation of its complexes with CDK4 and CDK6 complexes (cyclin D1/CDK4, cyclinD1/CDK6) and overcoming the G0/G1 checkpoint of the cell cycle (Ortiz et al. 2017). In the tested experimental conditions, an increased expression of the CCND1 due to glyphosate treatment at its whole tested range of the concentrations (0.5–100 μM) was identified, but no statistically significant changes in the methylation level of the gene promoter has been shown. Beside of changes in the expression level of the major cell cycle regulators and/or methylation pattern within their gene promoters, significant upregulation of the BCL2 expression at the mRNA level at the highest tested concentration of glyphosate (100 μM) was also identified. Therefore, this data may suggest a disturbance of apoptosis induction, due to sustained antiapoptotic abilities of BCL2. In contrast, *in vitro* studies on the effect of glyphosate on apoptosis in mature rat testicular cells (Clair et al. 2012) and PBMCs (Kwiatkowska et al. 2019) showed that glyphosate only at very high concentrations (29.57 mM/0.5 mM, in rat testicular cells and PBMCs; respectively) induced apoptosis. Thus, the obtained data may not result in specific metabolic effects until the exposure to high doses or cumulative long-term exposure of humans to glyphosate will be preceded.

Conclusion

This study has attempted to assess epigenetic mechanisms of action of glyphosate in human PBMCs, which has been poorly studied in cellular models including blood cells. The conducted analysis have shown that glyphosate significantly affected global DNA methylation of PBMCs as well as methylation in the promoter regions of selected tumor suppressors (P21 and TP53) as well as expression of major cell cycle and apoptosis drivers (P16, TP53, BCl2, CCND1 and P21). Changes in the DNA methylation profile were minimally correlated with gene expression level, however, regulation of transcription process is performed at many different levels and further and more global analysis (genome-wide based

on) are necessary to give clear answer about epigenetic-transcriptomic changes induced by glyphosate. It should be noted that glyphosate induced changes in concentrations corresponding to environmental or occupational exposure. Conducting of further *in vitro* studies on various epigenetic modifications caused by glyphosate with different target cell cultures is still warranted.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective was to assess epigenetic mechanisms of action of glyphosate in human PBMCs, which has been poorly studied in cellular models including blood cells. The study was conducted using an *in vitro* test system. The ability of glyphosate to impact the measured parameters was demonstrated (global DNA methylation of PBMCs, methylation in the promoter regions of selected tumor suppressors (P21 and TP53), and expression of major cell cycle and apoptosis drivers (P16, TP53, BCl2, CCND1 and P21). However, a positive control was not used, and a clear dose-response was not established for all of the measured parameters. Additionally, the measured effects *in vitro* are not clearly linked to an adverse outcome *in vivo*. While it is stated that the concentrations used are comparable to environmental exposure, external exposure was not linked to a corresponding internal concentration. Therefore, it is not possible to calculate a dose for risk assessment purposes. The study is useful for supplemental information on *in vitro* effects resulting from glyphosate exposure, but, is not appropriate for derivation of an endpoint in human health risk assessment.

This publication is considered relevant and reliable without restrictions because the assays conducted comply in general with the quality criteria for *in vitro* testing.

Reliability criteria for *in vitro* toxicology studies made by applicant

Publication: Wozniak *et al*., 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 95 %. Source: Sigma-Aldrich, USA.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	PBMC.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.5 to 100 µM
Cytotoxicity tests reported	Y	Ref. to earlier paper.
Positive and negative controls	Y	Methylated control DNA as positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication is considered relevant and reliable without restrictions because the assays conducted comply in general with the quality criteria for *in vitro* testing.		

Assessment and conclusion by RMS:
This public literature study is well described and shows sufficient details on the material and methods as well as the results. Overall, the study is concluded to reliable without restrictions based on the Klimisch score.

Similar to the applicant the RMS notes that for quite a few of the endpoints evaluated no dose-response relationship is observed despite the high spacing in the tested concentration. While the study gives some indication of a potential effect of glyphosate on methylation in the promotor region tumour suppressors *in vitro* it does not provide information on a potential adverse effect *in vivo*. Therefore, the study is considered to provide no information that will directly impact the risk assessment of glyphosate.

B.6.5.18.4. Supporting publications – Biserni 2019

Data point:	CA 5.5/029
Report author	Biserni, M. *et al.*
Report year	2019
Report title	Quizalofop-p-ethyl induces adipogenesis in 3T3-L1 Adipocytes
Document No	doi: 10.1093/toxsci/kfz097 ISSN: 1096-0929
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable without restrictions Conclusion AGG: Reliable without restrictions

Full summary of the study according to OECD format

In this study glyphosate, among other pesticide active substances, was investigated for its effect on lipid accumulation in differentiated adipocytes *in vitro* at concentrations ranging from 0.1 to 1000 µM. The results indicated that at the concentrations tested glyphosate scored negative for lipid accumulation.

Materials and methods
Chemicals – Glyphosate (purity ≥ 96 %) purchased from Sigma-Aldrich, Gillingham, UK. Stock solutions of glyphosate were prepared in serum-free medium and adjusted to pH 7.2.

Cell culture - The murine fibroblast 3T3-L1 cell line was purchased from ZenBio (Cambridge Bioscience, Cambridge, UK) and was not used past passage 10. Undifferentiated 3T3-L1 cells were grown at 37 °C under 5 % CO_2 in a maintenance medium composed of phenol red free Dulbecco's Modified Eagle Medium (DMEM), 10 % newborn calf serum, 2 mM glutamine and 10 µg/mL penicillin/streptomycin. Cells were released from the flask substratum using 0.05 % trypsin-EDTA and counted using a hemocytometer prior to seeding.

Adipocyte differentiation - For the differentiation of murine 3T3-L1 cell cultures to adipocytes, cells were seeded into 96-well plates at a density of 20,000 cells per well in 100 µL maintenance medium. Following a 2-day stabilization period, cells were switched to differentiation medium consisting of

DMEM, 2 mM glutamine, 10 µg/mL penicillin/streptomycin, 10 % fetal bovine calf serum, 500 µM 3-isobutyl-1-methyl-xanthine and 100 nM insulin from bovine pancreas. After a further 2 days of culture, the medium was refreshed to start the incubation with concentrations of glyphosate ranging from 0.1 to 1000 µM. Dexamethasone was used as a positive control. Media were replenished every 2 days for a further 6 days. Lipid accumulation was visualized on day 8 using fluorescent Nile Red staining in accordance with the manufacturer's instructions and quantified using a microplate reader. The fluorescence was measured with a filter giving an excitation at 490 nm and emission at 510-570 nm. The adipogenic effect was expressed as a fold change in emission signal intensity between untreated differentiated and treated differentiated 3T3-L1 cells.

Cell viability assay - Cell viability was assessed using a colorimetric 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, which indirectly measures cell number by testing for activity of mitochondrial succinate dehydrogenase. The 3T3-L1 cells were seeded into 96-well plates and differentiated as described above for 8 days. Cells were then incubated with 100 mL of MTT solution (1 mg/mL) for 2 hours and the test terminated by adding 100 µL DMSO. As a measure of cell number, the optical density of the cell lysate was determined at 570 nm using a GloMax Multi Microplate Multimode Reader. The number of cells was directly proportional to the intensity of the signal. Cell viability was expressed as a percentage of the control samples.

Intracellular lipid staining - The 3T3-L1 cells were seeded into 96-well clear bottom black tissue culture treated plates and differentiated as described above for 8 days. Medium was then removed and cells fixed by addition of 100 µL 4 % paraformaldehyde. Cells were then stained for intracellular lipid accumulation by adding 50 µL of 1 mg/mL Nile Red and 1 µg/mL 4',6-diamidino-2-phenylindole (DAPI) in 0.2 % Triton X-100-PBS for 15 minutes in the dark. Nile Red staining for lipid droplets and DAPI staining for cell nuclei were imaged at 530 and 405 nm, respectively, using fluorescence imaging on a Nikon Eclipse Ts2 microscope at 40 x.

Statistical analysis - The statistical analysis of the dose-response results from the adipogenesis assay was performed by ANOVA. Pair-wise comparisons were made using a Mann-Whitney test. Nonlinear regression analysis was performed using 5-parameter logistic dose-response curve models. These statistical analyses were performed using GraphPad Prism version 7.00 for MAC OS X.

Results

With dexamethasone as the positive control the adipogenic assay using murine 3T3-L1 cells to undergo differentiation to adipocytes was shown to be a sensitive assay system causing a maximum of 21-fold increase in lipid accumulation when compared to untreated differentiated cells. The dose response relationship of dexamethasone was used to determine the concentration that caused a 50 % response (EC_{50}). The EC_{50} for dexamethasone was 9.4 pM and some of the tested compounds showed an effect on lipid accumulation with different dose response patterns. Treatment with glyphosate scored negative.

Discussion

Commonly used herbicide active substances were tested in an adipogenesis assay to evaluate their obesogenic potential. Using the 3T3-L1 cell assay system, which measures lipid accumulation following differentiation to adipocytes, glyphosate scored negative at all concentrations tested. This study uses the well-established murine 3T3-L1 cell line model system for obesogenic screening. However, this cell line can only address a limited number of possible modes of action. The 3T3-L1 cells consist of unipotent pre-adipocytes, which can only differentiate into mature adipocytes. Another important limitation of 3T3-L1 cells is that they are of murine origin, and may not fully be representative of human metabolism. In addition, stocks of 3T3-L1 cells from different sources can have different metabolic capabilities.

Conclusion

Amongst the pesticide active substances tested for effects on lipid accumulation in differentiated adipocytes glyphosate scored negative.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study glyphosate, among other pesticide active substances, were investigated for their effect on lipid accumulation in differentiated adipocytes *in vitro* at concentrations ranging from 0.1 to 1000 µM. The results indicated that at the concentrations tested, glyphosate scored negative for lipid accumulation. This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.

Reliability criteria for *in vitro* toxicology studies made by applicant

Publication: Biserni *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≥ 96 %. Source: Sigma-Aldrich, Gillingham, UK
Only glyphosate acid or one of its salts is the tested substance	N	Other pesticide active substances were tested as well.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Concentration range *in vitro* from 0.1 to 1000 µM.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	Glyphosate was not tested in all tests described.
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	Y	No effect of glyphosate over the entire concentration range tested.
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Reliability not assignable		
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate and reliable without restrictions.		

Assessment and conclusion by RMS:
The study is considered to be well described in terms of method used and results observed. Overall, the study is concluded to be reliable without restrictions (Klimisch Score 1).

No effect of glyphosate on adipogenesis was observed.

B.6.5.18.5. Supporting publications – Crump 2019

Data point:	CA 5.5/030
Report author	Crump, K.
Report year	2019
Report title	The potential effects of recall bias and selection bias on the epidemiological evidence for the carcinogenicity
Document No	DOI: 10.1111/risa.13440 E-ISSN: 1539-6924
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, submitted for the purpose of the renewal
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable Conclusion AGG: Reliable.

Full summary of the study according to OECD format

The principal human data for glyphosate and non-Hodgkin's lymphoma (NHL) come from five case–control studies and two (related) cohort studies. The case–control studies are at risk of recall bias resulting from information on exposure to pesticides being collected from cases and controls based on their memories; cases being deemed likely by textbook authors to have a greater motivation than controls for remembering or reporting past exposures. In addition, two of the case–control studies are additionally at risk of a form of selection bias that can exacerbate the effect of recall bias. Both biases are in the direction of making glyphosate appear carcinogenic (viz. causing odds ratios (ORs) to be >1 in the absence of a true causal relationship). If ORs are not biased and a pesticide plays no role in causing NHL, the probability that an OR for that pesticide is greater than 1.0 is approximately 0.5. The fractions of ORs for pesticides other than glyphosate that are greater than 1.0 in the five case–control studies are 0.90 (n = 92), 0.90 (n = 152), 0.93 (n = 59), 0.76 (n = 140), and 0.53 (n = 54), the first two from studies that are at risk for both types of bias. In the two cohort studies, which are not subject to these biases, the comparable fractions for relative risks for all cancers are 0.51 (n = 70) and 0.48 (n = 158). Thus, this analysis provides evidence that at least four of the five case–control studies of glyphosate exposure and NHL are contaminated by statistical bias, likely stemming in the main from recall bias, exacerbated by selection bias in two of the studies. This suggests that these case control studies of glyphosate are not reliable evidence for a relationship between glyphosate and NHL.

Materials and methods

Two of the case–control studies exclude from some analyses of glyphosate (as well as analyses of other pesticides) the unexposed cases and controls who report exposures to pesticides not the subject of the analysis, which raises the possibility of selection bias if recall bias is also operating. The potential effect of selection bias in the presence of recall bias on ORs is illustrated by simulating sets of case–control data in which increasing amounts of recall bias are introduced, and the effects of adding selection bias to these analyses are noted. Each simulation involves 500 cases and 1,500 controls. Controls are randomly assigned exposure to pesticides according to the percentages of controls reporting exposure to nine pesticides reported by McDuffie *et al*. (2001) (Table II). Cases are randomly assigned exposures in the same way except the exposed percentages are multiplied by increasing factors in different simulations, which introduces increasing amounts of recall bias. In each of six simulations, 10,000 sets

of data are simulated and the 10,000 simulated ORs for glyphosate are averaged, (1): using all data so that recall bias but not selection bias is present and (2): after removing from the unexposed group both cases and controls that are exposed to any pesticide other than glyphosate (so that both recall bias and selection bias are present). In interpreting this simulation study, it needs to be understood that the McDuffie data and glyphosate are used only to make the simulations more realistic, and that the simulations say nothing about the McDuffie study or about the risk from glyphosate exposure, as any such data or a herbicide other than glyphosate could have been used to illustrate the same points.
The likelihood that statistical bias (from any source) may be responsible for the elevated ORs for glyphosate is evaluated by tabulating ORs and RRs from the studies and cross-classifying them by pesticide groups and the fraction that exceed 1.0. If all types of pesticides have an elevated percentage of ORs greater than 1.0, this suggests that bias may be the cause of the elevations rather than any carcinogenic effect of the pesticides.
The ORs and RRs included in the tabulations were selected from the original papers according to the following rules:

(1) In some instances, the exact same OR calculation is reported in two or more separate tables (e.g. the category "herbicide" in tables I, II, and V of Hardell *et al*., 2002). Only one of the identical calculations is tabulated.

(2) Similarly, some tables contain two sets of OR or RR calculations for testing the same hypotheses, but using different statistical methods (e.g. controlling for different sets of potential confounders). In these instances, only one set of calculations is tabulated, namely the set of ORs or RRs whose method of calculation agrees most closely with methods used in in the remainder of the article or in other articles. For example, De Roos *et al*. (2003) reported ORs calculated using both logistic regression and hierarchical regression. The ORs computed using logistic regression were selected for tabulation because this was the only study that employed hierarchical regression and logistic regression was the most common method used in the remaining studies.

(3) In De Roos *et al*. (2003), the category "potentially carcinogenic pesticides" apparently was formed post hoc and included those pesticides that gave greatest evidence of a carcinogenic effect in initial analyses. Such an approach would almost guarantee an OR greater than 1. In fact, the three ORs from this category were all greater than 1. ORs from this analysis were not included in order to avoid biasing the tabulation. (This does not imply that De Roos *et al*. erred in computing these ORs, only that they were not suitable for inclusion in our analysis.)

(4) Otherwise, all OR or RR calculations reported in the publications were tabulated. A complete listing of the ORs from each study contained in the tabulation is provided in the Supporting Information.

The results of these tabulations are summarized in graphs and in tabular form.

Results
Specific results part not given in this article. Results and discussion are merged.

Discussion
Results of the simulation exercise to demonstrate the effect of selection bias are shown in Table I. The first row in the table verifies that, as expected, selection bias does not affect the expected OR in the absence of recall bias. The remaining rows assume increasing amounts of recall bias as indicated in the first column. The effect of that recall bias on the expected ORs for glyphosate are shown in the second column. The third column shows the expected ORs when selection bias is added to the recall bias present by removing from the unexposed (to glyphosate) groups cases and controls exposed to any herbicide other than glyphosate, just as was done in Hardell *et al*. (2002) and Eriksson *et al*. (2008).

Table I. Results of Simulations[a] to Demonstrate the Effect of Recall Bias[b] Alone and with Selection Bias[c] on Average OR

Recall Bias[b]	Average OR for Glyphosate with Recall Bias Only	Average OR for Glyphosate with Both Recall Bias and Selection Bias
0	1.01	1.01
0.05	1.07	1.10
0.1	1.12	1.18
0.3	1.35	1.59
0.5	1.59	2.10
1	2.24	3.95

[a]10,000 simulations, 500 cases, 1,500 controls, each row. Herbicides assumed present and the percentage of controls assumed to report exposure: (glyphosate, 8%), (2,4-D, 19%), (mecoprop, 5.4%), (MCPA, 3.1%), (diclofopmethyl, 1.7%), (thiocarbamates, 3.3%), (bromoxynil, 3.2%), (dicamba, 8.7%), (dinitroaniline, 2.1%). Thus, for example, the probability that a particular control claimed exposure to MCPA was 0.031. Exposures to different herbicides are assumed to occur independently.
[b]Recall bias is introduced into each simulation by computing the prevalence of each herbicide exposure among cases as [prevalence among controls] × [1+ Recall bias]
[c]Selection bias is introduced by eliminating from OR calculations both unexposed (to glyphosate) cases and controls exposed to herbicides other than glyphosate.

Crump notes that the effect of selection bias increases with the increase in recall bias. This simulation demonstrates that selection bias can cause ORs to be inflated by important amounts above that due solely to recall bias when recall bias is also present.

Fig. 1 shows plots of the tabulated ORs or RRs by study, with the pesticide groupings upon which the ORs are based (including ORs derived for both individual pesticides and groups of pesticides), classified as to fungicides, herbicides not containing glyphosate, impregnating agents, insecticides, and pesticide groupings that include glyphosate. The individual pesticides and groupings for each study are listed in the footnote to Table II. The tabulated RRs from the two cohort studies are similarly classified into cancer groupings not containing NHL and groupings containing NHL. Since the logarithms of ORs are plotted, the focus is on the proportion of log-transformed ORs that are greater than 0.0 (equal to the proportion of untransformed ORs greater than 1.0).

These figures show that ORs in McDuffie *et al*. (2001), Hardell *et al*. (2002), and Eriksson *et al*. (2008) are nearly all greater than 1.0.

Table II. Counts of ORs and RRs Categorized by Study, Pesticide, and Whether Greater than 1.0

Case–Control Studies	Fungicides		Herbicides (No Glyphosate)		Impregnating Agents		Insecticides		Glyphosate-Containing		Glyphosate Only		Totals (No Glyphosate)		Totals (Incl. Glyphosate)	
	N	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1	*N*	% > 1
Hardell et al. (2002)[a]			31	93.5%	42	84.5%	19	94.7%	13	96.2%	1	100.0%	92	89.7%	106	90.6%
Eriksson et al. (2008)[b]	10	100.0%	44	92.0%	43	86.0%	55	89.1%	22	100.0%	11	100.0%	152	89.8%	185	91.6%
McDuffie et al. (2001)[c]	18	83.3%	19	94.7%			22	100.0%	6	100.0%	3	83.3%	59	93.2%	68	93.4%
Orsi et al. (2009)[d]	27	83.3%	36	72.2%			77	75.3%	27	66.7%	9	66.7%	140	76.1%	176	74.1%
De Roos et al. (2003)[e]			20	47.5%			34	55.9%	6	33.3%	1	100.0%	54	52.8%	61	51.6%
Totals	55	86.4%	150	82.0%	85	85.3%	207	80.2%	74	81.8%	25	86.0%	497	82.3%	596	82.4%

Cohort Studies	No NHL		NHL + Subtypes		Totals	
	N	% > 0	*N*	% > 0	*N*	% > 0
De Roos et al. (2005)[f]	55	53.6%	15	43.3%	70	51.4%
Andreotti et al. (2018)[g]	86	51.2%	72	43.1%	158	47.5%
Totals	141	52.1%	87	43.1%	228	48.7%

[a]ORs for NHL and hairy cell leukemia computed for herbicides; phenoxyacetic acids; MCPA; 2,4,5-T + 2,4-D; glyphosate; other herbicides; insecticides; DDT; mercurial seed dressing; pyrethrins; fungicides; impregnating agents; chlorophenols; pentachlorophenol; arsenic; creosote; other impregnating agents; organic solvents.

[b]ORs for NHL, including subtypes, as well as several subcategories of NHL were computed for herbicides, total; phenoxyacetic acids; MCPA; 2,4,5-T and/or 2,4-D; other phenoxyacetic acids; herbicides except phenoxyacetic acids; glyphosate; other herbicides; insecticides, total; DDT; mercurial seed dressing; pyretrine; permetrine; other insecticides; fungicides; impregnating agents; chlorophenols; arsenic; creosote; tar; other impregnating agents; rodenticides.

[c]ORs for NHL computed for phenoxyherbicides; 2,4-D; mecoprop; MCPA; diclofopmethyl; phosphonic acid; glyphosate (Roundup); thiocarbamates; diallate; phenols: bromoxynil; dicamba; dicamba (Banvel or Target); dinitroaniline; trifluralin; carbamates; carbaryl; carbofuran; methomyl; organochlorine; chlordane; lindane; aldrin; organochlorine diphenylchlorides; DDT; organophosphorus; malathion; dimethoate; diazinon; amide; captan; vitavax; aldehyde; formaldehyde; mercury containing; mercury dust; mercury liquid; sulfur compounds.

[d]ORs for NHL, including subtypes, and other categories of lymphoid neoplasms computed for occupational pesticide use, insecticides, organochlorine, organophosphate, pyrethrin, fungicides, carbamates, imide, triazole, herbicides, phenoline, phenoxy, picoline, triazine, amide, urea, quaternary ammonium, glyphosate, garden pesticide use, insecticides, fungicides, herbicides, domestic insecticide use.

[e]ORs for NHL computed for aldrin; bufencarb; carbaryl; carbofuran; chlordane; copper; acetoarsenite; coumaphos; DDT; diazinon; dichlorvos; dieldrin; dimethoate; ethoprop; famphur; fly, lice, or tick spray; fonofos; heptachlor; lead; arsenate; lindane; malathion; methoxychlor; nicotine; phorate; pyrethrins; rotenone; tetrachlorvinphos; toxaphene; terbufos; alachlor; atrazine; bentazon; butylate; chloramben; cyanazine; 2,4-D; dicamba; EPTC + protectant; glyphosate; linuron; MCPA; metolachlor; metribuzen; paraquat; propachlor; sodium; chlorate; 2,4,5-T; trifluralin; any pesticide; any insecticide; any herbicide; chlordane and DDT; carbofuran and atrazine; diazinon and atrazine; alachlor and atrazine; atrazine and dicamba.

[f]RRs computed for glyphosate exposure in relation to the following cancers: all cancer, lung, oral cavity, rectum, pancreas, kidney, bladder, prostate, melanoma, lymphohematopoietic cancer, NHL, lukemia, and multiple myeloma.

[g]RRs computed for glyphosate exposure in relation to the following cancers: all cancer; oral cavity; colon; rectum; pancreas; lung; melanoma; prostate; testicular; bladder; kidney; lymphohematopoietic; Hodgkin lymphoma; non-Hodgkin lymphoma; non-Hodgkin lymphoma B cell; chronic lymphocytic lymphoma or small lymphocytic leukemia; diffuse large B cell lymphoma; marginal-zone lymphoma; follicular lymphoma; multiple myeloma and non-Hodgkin lymphoma T cell.

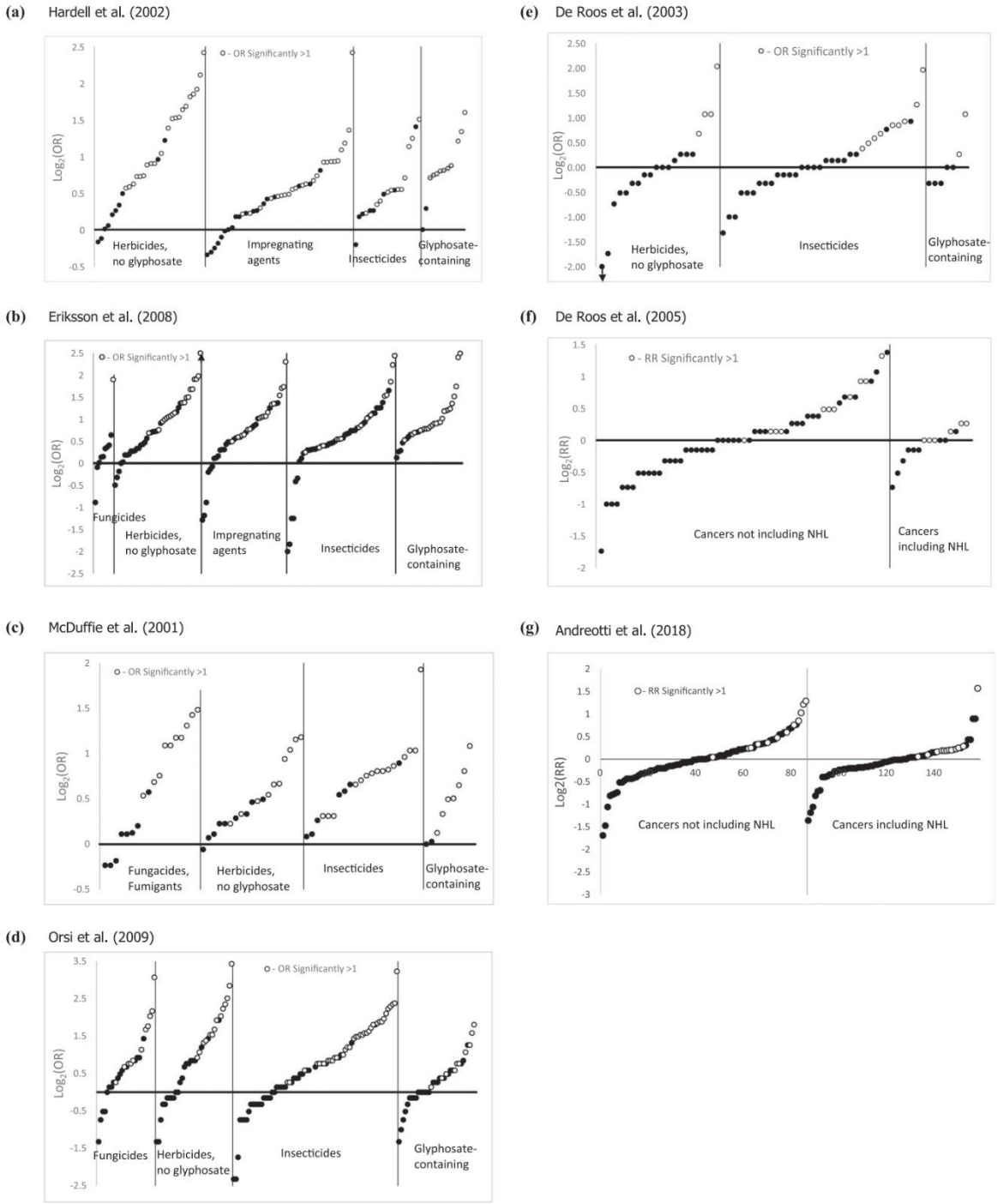


Fig. 1. Graphs of tabulated ORs and RRs. (a) Hardell et al. (2002), (b) Eriksson et al. (2008), (c) McDuffie et al. (2001), (d) Orsi et al. (2009), (e) De Roos et al. (2003), (f) De Roos et al. (2005), (g) Andreotti et al. (2018).

Also, there is an excess of ORs greater than 1.0 in Orsi *et al*. (2009). These excesses of ORs greater than 1.0 occur in all categories of pesticides considered in these studies. On the other hand, there appears to be roughly a balance between the numbers of ORs greater than and less than 1.0 in all categories of pesticides in the case–control study of De Roos *et al*. (2003). Similarly, in both cohort studies (Andreotti *et al*., 2018; De Roos *et al*., 2005), there seems to be roughly an equal number of RRs greater than and less than 1.0, both for cancer groupings that include NHL and those that do not.

These graphs also display those ORs and RRs that are statistically significantly greater than 1.0, with statistical significance defined by the lower bound on the 95 % confidence interval being greater than

or equal to 0.8 (apparently the decision rule used in De Roos *et al*., 2003 to define "potentially carcinogenic pesticides.") This shows that many statistically significantly elevated ORs occur in every pesticide category with little notable difference between categories that contain glyphosate and those that do not.

Table II, which summarizes the tabulated ORs and RRs, provides confirmation of the impressions obtained from the graphs. In this table OR and RR reported in the original papers as equal to 1.0 (which are mostly due to roundoff in reported values) each contribute 0.5 to the counts of ORs and RRs greater than 1.0. In the case–control studies of Hardell *et al*. (2002), Eriksson *et al*. (2008), and McDuffie *et al*. (2001), 90 % or more of all ORs from pesticide groups not containing glyphosate are greater than 1.0. In these three studies, the percentage of ORs greater than 1.0 exceeds 80 % in all pesticide groupings (fungicides, herbicides not including glyphosate, impregnating agents, and insecticides, as well as groupings that contain glyphosate). The percentages of ORs from Orsi *et al*. (2009) that exceed 1.0 are also elevated, although not to the same extent as in the other three studies. By contrast, the percentages of ORs from the case–control study of De Roos *et al*. (2003) that are greater than 1.0 are all fairly close to 50 % in all pesticide categories (52.8 % in categories combined than do not include glyphosate and 51.6 % if glyphosate-containing categories are included). Thus, of the five case control studies, the study of De Roos *et al*. (2003) presents considerably less evidence of recall bias resulting from an excess of ORs greater than 1.0.

In the two cohort studies, the percentages of RRs greater than 1.0 in cancer groupings not containing NHL in both studies are 54 %, and overall, with NHL included, are 49.5 % (Table II). Thus, these results from the two cohort studies, which are not subject to recall bias or selection bias, are reasonably consistent with what would be expected if these studies are free of statistical bias and glyphosate has no effect upon cancer rates.

If the ORs are not biased, the results in Table II pertaining to the case–control studies suggest that all types of pesticides investigated in these studies are having a role in causing NHL, including fungicides, herbicides other than glyphosate, impregnating agents and insecticides. It should also be kept in mind that the category NHL contains many types of lymphoma, not all of which are likely to share common risk factors. Thus, it seems unlikely (at least to this investigator) that pesticides within each of these types of pesticides would be causing NHL, and particularly to an extent to be responsible for the evidence seen in Table II. It seems much more likely that the preponderance of ORs greater than 1.0 seen in all pesticide categories in most of the case–control studies is simply the result of recall bias, which is a well-known problem with these types of case–control studies, possibly augmented in two studies by selection bias. Such a conclusion is further supported by the fact that in the two cohort studies, which are not subject to these biases, the overall percentage of RRs greater than 1.0 is 49.6 % that is in excellent agreement with the theoretical value of 0.5, assuming no bias and no effect of glyphosate on any cancer.

Given this evidence, one could reasonably conclude that at least four of the case–control studies of glyphosate and NHL are contaminated by statistical bias, and consequently are not suitable for reaching conclusions about the potential ability of glyphosate to cause NHL.

The potential for case–control studies to be affected by recall bias is well known and has been discussed in many publications. The potential for the case–control studies of glyphosate, in particular, to be subject to recall bias, along with concerns about glyphosate studies not controlling for exposure to farm animals, and for the use of proxy respondents were discussed previously. These same issues were raised by some panellists in an EPA FIFRA scientific advisory panel.

Conclusion

In summary, the potential for these types of case–control studies to be contaminated by bias from the use of exposure information based on the memories of both cases and controls (recall bias) is well known. This article provides evidence that at least four of the five case–control studies of glyphosate exposure and NHL are contaminated by statistical bias, likely stemming in the main from recall bias, exacerbated by selection bias in two of the studies. This suggests that the case control studies of glyphosate are not reliable for determining whether glyphosate is carcinogenic. However, the two cohort studies (Andreotti *et al*., 2018; De Roos *et al*., 2005) do not present evidence of bias. If further study of the potential relationship between glyphosate exposure and NHL is needed, it would best come from

cohort or other studies that are not at risk of recall bias resulting from quantifying exposures by questioning subjects. Of course, cohort studies have other potential problems that must be evaluated, including incomplete follow-up, the healthy worker effect and poor information on exposures. The potential for recall bias identified herein could affect, not just case–control studies of the potential carcinogenicity of glyphosate, but any such study that involves quantifying exposures occurring in the distant past based on participant's memories.

Assessment and conclusion

Assessment and conclusion by applicant:

It is well known that recall bias is a potentially important bias in cancer case control studies where study participants are asked to recall their past exposures. In an ideal study, information about exposures for cases and controls would be collected under exactly the same circumstances. However, circumstances are quite different for cases and controls. Cancer cases have suffered a grievous illness and it is only natural for them to be deeply introspective about what might have caused their cancers. Controls have no such motivation that would augment their recall (or reporting). So, the concern expressed in many textbooks is that recall bias tends to produce false positive results. The purpose of this analysis by Crump was to evaluate the evidence for recall bias in the overall pattern of results in five case control studies and two cohort studies that comprise the main part of the glyphosate-NHL literature.

In evaluating the case control studies, Crump reasoned that the percentage of odds ratios > 1 for non-glyphosate exposures should be approximately 50 % if recall bias was not operative and those exposures did not cause NHL. Yet, it turned out that the percentages of ORs >1 for non-glyphosate exposures were 90 % for Hardell *et al*. (2002), 90 % for Erikson *et al*. (2008), 93 % for McDuffie *et al*. (2001), 76 % for Orsi *et al*. (2009), and 53 % for DeRoos *et al*. (2003). These extreme departures from 50 % for 4 of the 5 case control studies is consistent with recall bias, perhaps augmented by a type of selection bias in the analyses by Hardell *et al*. (2002) and Eriksson *et al*. (2008). In contrast, in the most recent publication from the Agricultural Health Study (Andreotti *et al*. 2018), only 48 % of the relative risks (RR) calculated were >1 – a percentage in the range expected with a true probability of 50 %. While the evaluation of Andreotti *et al*. (2018) concerned glyphosate and other cancer sites and not other exposures and NHL, the principle is the same: under the null hypothesis the proportion of ORs or RRs > 1 should be roughly 50 % absent bias.

We agree with Crump's conclusion that the 4 case-control studies with a high proportion of ORs > 1 are "contaminated" by statistical bias and are not reliable as evidence of a relationship between glyphosate and NHL. Of course, there are also other types of bias that may contribute to the high proportion of positive ORs (e.g. lack of control for confounding, lower participation for controls than cases (traditional selection bias), proxy respondents, etc.) (see Acquavella *et al*. 2016). Nonetheless, Crump's point is well taken that ORs for glyphosate in 4 of the 5 case control studies should be interpreted as unreliable because the vast majority of ORs for other exposures are >1.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Crump K., 2019	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	For a methodologic evaluation of recall bias in existing studies
Appropriate study population to address potential glyphosate-related health outcomes	Not applicable	
Exposure studied		
Exposure to formulations with glyphosate as a.s.	Yes	

Publication: Crump K., 2019	**Criteria met? Y/N/?**	**Comments**
Exposure to formulations with other a.s.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Not applicable	
Adequate description of exposure circumstances	Not applicable	
Comparable participation by groups being compared	Not applicable	
Information provided by proxy respondents	Not applicable	
Adequate statistical analysis	Yes	To illustrate bias
Adequate consideration of personal confounding factors	Not applicable	
Adequate consideration of potentially confounding exposures	Not applicable	
Overall assessment		
Reliable without restrictions	Yes	As methodologic work. Clearly illustrates recall bias in the glyphosate case control studies.
Reliable with restrictions	No	
Not reliable	No	

Assessment and conclusion by RMS:

The study provides on analysis of the ORs obtained in the epidemiological studies for glyphosate with the aim to evaluate the potential of recall bias. Based on the high percentage of ORs above 1 it seems that recall bias may have played a factor in a number of the case-control studies.

Full evaluations of each epidemiological studies are conducted elsewhere in the RAR.

B.6.5.18.6. Supporting publications – Duforestel, 2019

Data point:	CA 5.5/031
Report author	Duforestel, M. *et al.*
Report year	2019
Report title	Glyphosate primes mammary cells for tumorigenesis by reprogramming the epigenome in a TET3-dependent manner
Document No	doi: 10.3389/fgene.2019.00885 ISSN: 1664-8021
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities

Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable, the only limitation noted was the missing information regarding the impurity of the test material.

Full summary of the study according to OECD format

The acknowledgment that pollutants might influence the epigenome raises serious concerns regarding their long-term impact on the development of chronic diseases. The herbicide glyphosate has been scrutinized for an impact on cancer incidence, but reports demonstrate the difficulty of linking estimates of exposure and response analysis. An approach to better apprehend a potential risk impact for cancer is to follow a synergistic approach, as cancer rarely occurs in response to one risk factor. The known influence of glyphosate on estrogen-regulated pathway makes it a logical target of investigation in breast cancer research. In this study, non-neoplastic MCF10A cells in a repeated glyphosate exposure pattern over 21 days were used. Glyphosate triggered a significant reduction in DNA methylation, as shown by the level of 5-methylcytosine DNA; however, in contrast to strong demethylating agent and cancer promoter UP peptide, glyphosate-treated cells did not lead to tumor development. Whereas UP acts through a DNMT1/PCNA/UHRF1 pathway, glyphosate triggered increased activity of ten-eleven translocation (TET)3. Combining glyphosate with enhanced expression of microRNA (miR) 182-5p associated with breast cancer induced tumor development in 50 % of mice. Culture of primary cells from resected tumors revealed a luminal B (ER+/PR-/HER2-) phenotype in response to glyphosate-miR182-5p exposure with sensitivity to tamoxifen and invasive and migratory potentials. Tumor development could be prevented either by specifically inhibiting miR 182-5p or by treating glyphosate-miR 182-5p-cells with dimethyloxallyl glycine, an inhibitor of TET pathway. Looking for potential epigenetic marks of TET-mediated gene regulation under glyphosate exposure, we identified MTRNR2L2 and DUX4 genes, the hypomethylation of which was sustained even after stopping glyphosate exposure for 6 weeks. The findings reveal that low pressure but sustained DNA hypomethylation occurring *via* the TET pathway primes cells for oncogenic response in the presence of another potential risk factor. These results warrant further investigation of glyphosate-mediated breast cancer risk.

Materials and methods

Cell Culture and Transfection; MCF10A cells were cultured in DMEM/F12 supplemented with 5 % horse serum (Invitrogen, Cergy Pontoise, France), 500 ng/ml hydrocortisone (Sigma-Aldrich, France), 100 ng/ml cholera toxin (Sigma-Aldrich, France), 10 µg/ml insulin (TermoFisher, France) and 20 ng/ml epidermal growth factor (EGF, SigmaAldrich, France), penicillin (100 U/ml), and 2 mmol/L L-glutamine. MCF7 and MDA-MB-231 cells were cultured in DMEM medium (Invitrogen) all supplemented with 5 % FCS and 2 mM l-glutamine. Glyphosate (CAS 1071-83-6, sc-211568) was purchased from Santa-Cruz (France), and a 10^{-8}-M stock solution was prepared in DMSO every week. Glyphosate was diluted directly in fresh cell culture medium and was fed to the cells at the time points indicated in the results section. For the transfection of RNAs, we used miRCury LNA miR mimics for the has-miR-146a, has-miR-182-5p, has-miR-27a, has-miR-500a-5p, has-miR-30a, and has-miR-495 (Qiagen, France), siRNA for siRNA-T ET3 (sc94636) and control siRNA-A (sc94636) and HIPerfect Transfection Reagent (Qiagen, France). All miRs showed similar transfection efficiency (10- to 15-fold change, as measured by RTqPCR).

DNA Extraction, 5mC ELISA, and qMSRE; A QIAcube automate and QIAmp DNA Mini QiaCube kit (Qiagen, France) were used to isolate DNA. The quantification of 5mC was performed using the 5mC DNA ELISA Kit (Zymo Research-Euromodex, France) according to the manufacturer's instructions. The 5mC DNA ELISA Kit estimates the number of 5mC on DNA without distinction of localization; therefore, the term of global DNA methylation level when referring to results obtained *via* this mode of quantification was used. Next, DNA methylation was quantified by qMSRE. Digestions were performed with adequate restriction enzymes, HpaII and AciI (NEB, France). Typically, 1 ng of genomic DNA

was digested with 40 U of enzymes at 37 °C for 2 h in 50 µl of reaction. Control samples were treated in the same way but without addition of the enzyme. Five microliters of digestion mixture were used for qPCR. The QuantiFast SYBR Green PCR Kit and Rotor-Gene Q (Qiagen, France) were used to perform the qPCR. Primers were MSH3: TTTCTCCAG GGCTGGGACTTTG and CCCGACTGGATTCCCCTTTTCT; DHFR: AAACCTCAGCGCTTCACCCAAT and TGATAGG GCTGGAGGAGGAAG; DUX4: CGACACCCTCGGACAGCA and TCAAAGCAGGCTCGCAG; COL23A1: TCTCCAGG CCAGAAACAGTCTT and ATTTAGAGAGGCAGGGTC GAGA; and MTRNR2L2: ACCCCACCTGTTTACCAA and GCTACCTTTGCACGGTTAGGG.

Tumor Xenografts in Nude Mice; Cells were harvested by trypsinization, washed and resuspended in saline buffer. Cell suspensions were injected subcutaneously into the flank of 7 to 8-week-old mice (Janvier, France) in 100 µl of sterile PBS. Tumor volume based on caliper measurements was calculated using the ellipsoidal formula [Tumor volume = 1/2 (length × width^2)] according to previously published work. At the end of the observation period, the mice with xenograf tumors were euthanized, and the tumor tissues were removed for analysis. The experimental procedures with animals were in accordance with the guidelines of Institutional Animal Care and the French National Committee of Ethics. In addition, all experiments were conducted according to the Regulations for Animal Experimentation at the Plateforme Animalerie in the Institut de Recherche en Santé de l'Université de Nantes (IRS-UN) and approved by the French National Committee of Ethics. The number of mice was restricted to four per condition to limit the number of animals to the necessary minimum as in previous studies based on the fact that we anticipated to detect a highly frequent tumorigenic event (frequency superior to one to four for tumorigenesis).

Establishment of Tumor Cells; From Xenografts (PCTCdX); PCTCdX (here named Glypho-iBPCTC) were obtained after mechanical dissociation. Briefly, resected tumor tissue from mice was cut into pieces of 1–5 mm^3 and plated in a 60-mm^2 tissue culture dish with DMEM containing 10 % FBS and antibiotics. Minced pieces of tumor were incubated with 200 U/ml collagenase I (Sigma) and 500 U/ml DnaseI (Sigma) in PBS for 1 h at 37 °C with vigorous constant agitation. The single cell suspension was filtered through a 70-mm cell strainer (BD Falcon), washed with PBS, and then placed in DMEM-10 % FBS. Cell cultures were split 1:5 when confluent.

Migration Assay; Cells (3×10^5) were seeded in six-well plates, cultured until they reached 80–90 % confluence, and treated with 10 µg/ml of mitomycin C (Sigma, France) for 2 h (to prevent cell proliferation). The monolayer of cells was scratched using a two-well silicone insert (Ibidi, Germany). Cell migration was monitored by microscopy (Incellis Cell Imager, Bertin, France). The images acquired at different time points (0, 4, 8, 24, 28, 32, and 48 h) for each sample were analyzed quantitatively. For each image, distances between one side of the wound and the other side were measured with ImageJ software; the mean value of 10 measurements all along the wound was recorded. The average migration speed was obtained by calculating the ratio distance/time along the time course.

Invasion Assay; All of the procedures were performed according to the manufacturer's instructions (QCM 24-Well Collagen-Based Cell Invasion Assay, Millipore, France). In brief, 200 µl of serum-free medium containing 2×10^5 cells were added into the invasion chamber, with the bottom well of the 24-well plate containing 500 µl of complete medium. After 72 h of incubation at 37 °C, the medium was removed, and the cells were stained by placing the chamber in staining solution for 20 min at room temperature. Cells that did not invade were carefully removed from the top side of the chamber using a cotton swab. The stained chamber was inserted into a clean well containing 200 µl of extraction buffer for 15 min at room temperature. A total of 100 µl of extracted (stained) solution from the chamber was transferred into a 96-well plate, and the optical density was measured 570 nm using a spectrophotometer.

Viability Assay: MTT and XTT Tests; A cell suspension containing 10^5 cells was prepared, and 100 µl was distributed in sixplicates in a 96-well plate. After 24 h of incubation at 37 °C and 5 % CO_2, cells were exposed to tamoxifen for 48 h. Tamoxifen was first diluted 10 times in dimethyl sulfoxide (DMSO)

and then further diluted in DMEM containing 4.5 g/L glucose, 1 % SVF, 1 % glutamine, 1 % penicillin-streptomycin at the desired concentrations. Following treatment, 10 µl of MTT (10 µg/ml) (VWR Chemicals, France) was added in each well, and the cells were incubated for 3 h. Finally, the medium containing MTT was removed, and 200 µl/well of DMSO was added to measure the optical density at 570 nm using a spectrophotometer. For the XTT test, the XTT Assay Kit was used (ab232856, Abcam, France) according to the manufacturer's instructions. Briefly, 10^5 cells were seeded in 100 µl of culture medium in each well of a 96-well plate. After 24 h of incubation at 37 °C and 5 % CO_2, cells were treated with adequate drugs. Then, 10 µl/well of XTT mixture was added for an incubation of 2 h at 37 °C and 5 % CO_2. Finally, absorbance was measured at 450 nm.

Breast Tissue and Urine Samples; Human samples were collected from the Réseau des tumorothèques du Cancéropole Grand-Ouest and Institut de Cancérologie de l'Ouest. In accordance with regulations, all subjects signed a specifc informed consent form for this biocollection approved by an Ethics Committee (CPP OUEST IV, n °18/16), the French State Department for National Education, Higher Education and Research (Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche, N ° DC-2015-2457) and the Commission Nationale de l'Informatique et des Libertés (CNIL) (compliance commitment to MR 001). The glyphosate concentration in urine samples was obtained using Glyphosate kit (Novakits, France).

mMTase and TET Activities; TET activity was determined using the Epigenase 5mC-Hydroxylase TET Activity/Inhibition Assay Kit (Colorimetric; Epigentek/Euromedex, France) according to the manufacturer's instructions. Dnmts-magnetic beads (DMB) assays were performed to estimate mMTase, such as initially described. Briefly, a typical methylation reaction required 50 µg of nuclear extract (Nuclear extract kit, Active Motif, France), 125 nM DNA oligonucleotides (probes), and 900 nM tritium-labeled AdoMet (1 mCi/ml; #NET155V001MC; PerkinElmer, France) in reaction buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 10 % glycerol, 0.5 mM phenylmethylsulfonyl fluoride). After incubation at 37 °C for 1 h, reactions were quenched with an equal volume of magnetic beads suspension and incubated for 15 min at room temperature. Next, the beads were magnetically isolated from the reaction mix, and tritium incorporation was measured by scintillation counting.

In-Cell ELISA; In-cell ELISA was performed using the In-Cell ELISA Kit (Abcam, France) according to the manufacturer's instructions and after a fixation step performed with 4 % of paraformaldehyde solution (10 min at room temperature). Primary antibodies were incubated overnight at 4 °C. Adequate HRP-conjugated secondary antibodies were incubated for 1 h at room temperature. Detection was performed at 450 nm. After the washes, cells in each well were incubated with 1X Janus Green Stain for 5 min at room temperature, according to the manufacturer's instructions. Data were expressed in normalized unit, according to the following calculation: (HRPsignal 'minus' HRPsignal in absence of primary antibody)/(Janus Green signal 'minus' Janus Green signal in absence of cells). Antibodies used were anti-TET1 (sc163446, Santa Cruz, France), anti-TET2 (sc398535, Santa Cruz), anti-TET3 (sc139186, Santa Cruz), anti-Erα (sc8002, Santa Cruz), anti-PR (sc130071, Santa Cruz), and anti-HER2 (sc-393712, Santa Cruz).

ChIP Analyses; ChIP was performed using the ChIP-IT Express kit (Active Motif, France) according to the manufacturer's instructions. The cross-linking step was performed by treating the cells with 37 % formaldehyde solution for 15 min at room temperature. Sonication was performed with the Bioruptor Plus (eight cycles 30 s on/90 s off) (Diagenode, France). The QuantiFast SYBR Green PCR Kit and Rotor-Gene Q (Qiagen, France) were used to perform the qPCR. Antibodies used were Anti-IgG (Abcam, AB2410) and anti-TET3 (sc139186, Santa Cruz).

Statistical Analysis; All experiments were done at least in biological triplicates. Differences in means were assessed using Student t test, and the degree of correlation between two parameters was calculated using Pearson's test. $P < 0.05$ was considered significant.

Results

Exposure to Glyphosate Promotes TET3-Mediated Global DNA Hypomethylation in MCF10A Cells; DNA hypomethylation has been shown to play a determining role in cancer development. To verify the impact of glyphosate exposure on the global level of DNA methylation, non-neoplastic breast epithelial MCF10A cells were treated with a low dose (10-11 M) of this herbicide every three to four days over 21 days, covering three passage numbers; whereas control cultures were treated with vehicle DMSO (Figure 1A). Several articles analyzing the effect of glyphosate on human cells have reported using 10-11 M. Indeed, 90 % of MCF10A cells were viable as measured by XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay at this concentration. Importantly, glyphosate 10-11 M is below the concentration detected in biological fluids (milk, serum, urine). As a control performed in parallel, MCF10A cells were exposed to carcinogenic UP peptide (0.5 μM) previously described to promote global DNA hypomethylation *via* the disruption of the DNMT1/PCNA/UHRF1 complex. As expected, there was a decrease in the level of 5mC-DNA in MCF10A cells treated with the UP peptide (Figure 1B). There was also a reduction in 5mC content in cells treated with glyphosate (Figure 1B), hence suggesting that glyphosate promotes a global DNA hypomethylation as per the definition given in the introduction. The origin of glyphosate-mediated decrease in DNA methylation was assessed by measuring the levels of activity of maintenance methyltransferase (mMTase) and Ten-eleven translocation (TET), since a decrease of mMTase activity and an increase of TET activity are both causes of DNA hypomethylation. The mMTase activity remained unchanged in MCF10A cells treated with glyphosate (Figure 1C) while TET activity significantly increased in these cells (Figure 1D). Specifically, an ELISA-based assessment of the amount of the three TET family members, TET1, TET2 and TET3, revealed an overexpression of TET3 in MCF10A cells following exposure to glyphosate (Figure 1E). To confrm that glyphosate promotes TET3-mediated global DNA hypomethylation in MCF10A cells, we analysed the level of DNA methylation in MCF10A cells with siRNAmediated TET3 down-regulation. ELISA results show that the presence of siRNA-TET3 abrogates TET3 overexpression and prevents DNA hypomethylation in cells exposed to glyphosate (Figure 1F).

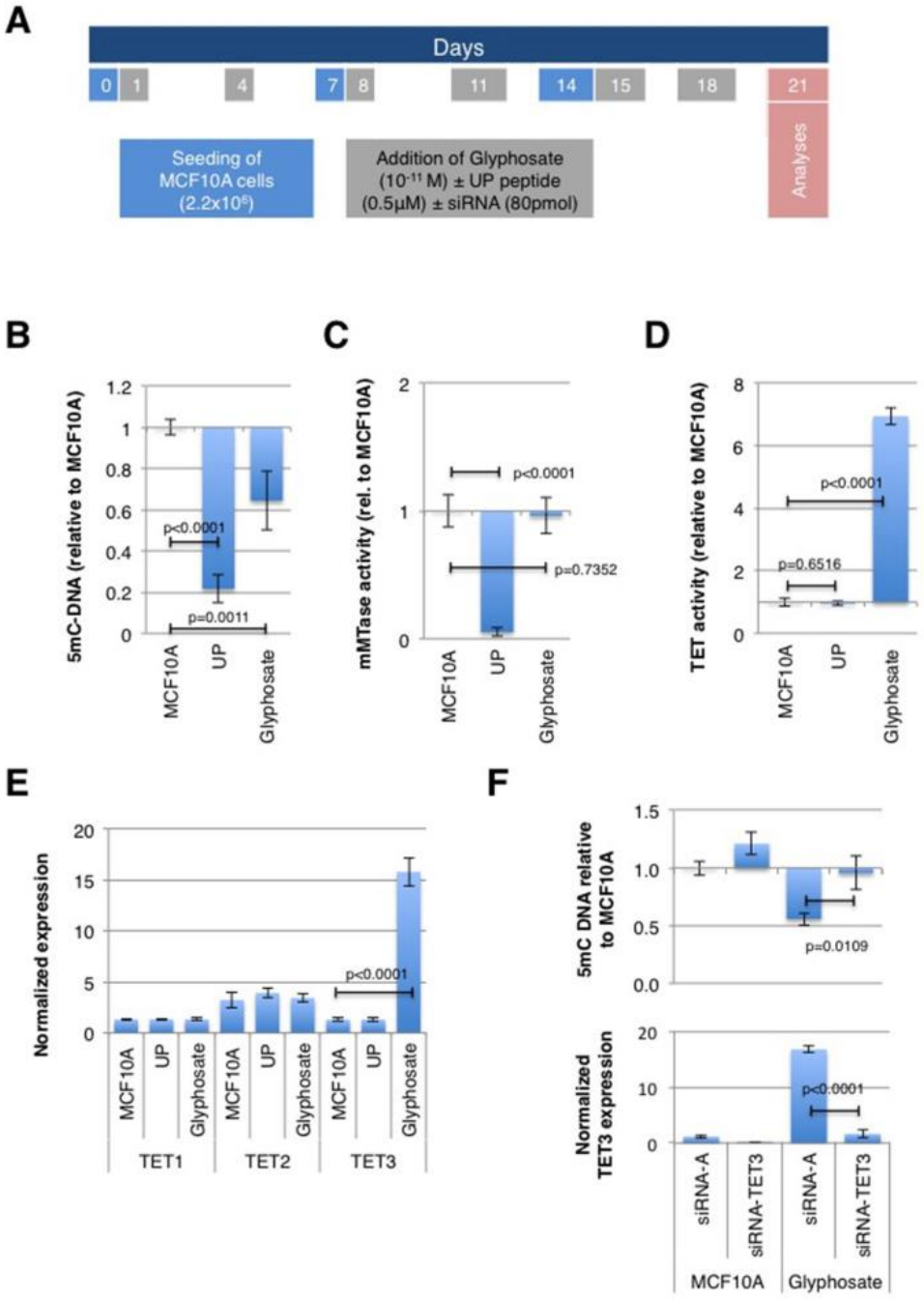


FIGURE 1 | Glyphosate exposure promotes a TET3-mediated global DNA hypomethylation. MCF10A cells were treated according to a timetable shown in **(A)** and analyzed on day 21 of culture. (Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. UP peptide promotes DNMT1/PCNA/UHRF1 disruption). **(B)** ELISA was used to measure the level of 5-methylcytosine (5-mC). **(C)** DMB assay was used to measure maintenance methyltransferase (mMTase). **(D)** TET assay. **(E)** In-Cell ELISA was used to quantify TET proteins. **(F)** MCF10A cells were transfected either with siRNA for TET3 or with control siRNA (siRNA-A) and treated with glyphosate (Glyphosate) or vehicle DMSO (MCF10A) according to a timetable shown in **(A)**. ELISA was used to measure the level of 5mC, and TET3 levels were determined by In-Cell ELISA and normalized to Janus Green staining intensity to account for differences in cell seeding density. For all assays, the bar graph displays the average ± standard deviation values of three independent experiments.

Glyphosate Exposure Is Tumorigenic for MCF10A Cells in a Two-Factor Hit Model; Global DNA hypomethylation is potentially tumorigenic. Therefore, MCF10A cells exposed to glyphosate were injected subcutaneously in Swiss nude mice. No tumors developed, whereas the control experiment with MCF10A cells exposed to the UP peptide led to visible tumor growth within 21 days in 100 % of the mice (Figure 2A). The Knudson's hypothesis for cancer initiation suggests that several oncogenic hits cooperate to promote cancer. This hypothesis initially based on mutations can be transposed to risk

factors beyond genetic alterations. Indeed, several microRNAs (miR) have been associated with cancer either as oncomiR (one hit) or suspected to promote cancer phenotype in light of their overexpression in cancers. To investigate the possibility of a two factor hit oncogenic impact with glyphosate, six miRs associated with poor prognosis of breast cancer [miR-182-5p, miR-27a, miR-500a-5p, miR-30a, miR-495, and miR-146a] were transfected individually in MCF10A cells. For this purpose, miRs mimics were used, and their increased expression was confirmed by RTqPCR. Tumor nodules were observed in two out of the four mice with subcutaneous injection of glyphosate-exposed MCF10A overexpressing miR-182-5p, whereas none of the other five miRs were associated with tumor formation (Figure 2B). Moreover, no tumor nodules were observed with subcutaneous injection of glyphosate/miR-182-5p/siRNA-TET3-exposed MCF10A, confirming that TET3 is implicated in glyphosate-mediated tumorigenic pathway (Figure 2C). The use of the Pan-cancer RNA-seq data available from the KM plotter database revealed that although TET3 overexpression is associated with a favorable overall survival in head and neck squamous cell carcinoma, thymoma, and thyroid carcinoma, it is associated with an unfavorable overall survival in breast cancer, as well as cervical squamous cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, pheochromocytoma, paraganglioma, and uterine corpus endometrial carcinoma. We next compared several molecular signatures and phenotypic traits of primary cultures of tumor cells (PCTC) from glyphosate-induced breast tumors (Glypho-iBPCTC) with the ones of luminal A (MCF-7) and triple negative (MDA-MB-231) breast cancer cells. Only one of the two tumors led to viable Glypho-iBPCTC. In-cell ELISA confirmed that MCF7 and MDA-MB-231 cells were Erα+/PR+/HER2- (luminal A) and Erα-/PR-/HER2- (triple negative), respectively, and revealed that Glypho-iBPCTC were Erα+/PR-/HER2-, hence corresponding to a luminal B type of breast cancer with poorer outcome compared to ER+/PR+/HER2- subtype (Figure 3A). Tamoxifen/IC50 in MCF-7 and Glypho-iBPCTC were similar (Figure 3B). The QCM™ 24-Well Collagen-based cell invasion assay revealed that all cell strains had similar invasion capacity (Figure 3C), although scratch test indicated that Glypho-iBPCTC had the lowest migration ability compared to MCF-7 ($p = 0.0137$) and MDA-MB-231 cells ($p = 0.0002$) (Figure 3D). These results confirm that Glypho-iBPCTC display phenotypic traits associated with breast cancer cells *in vitro*.

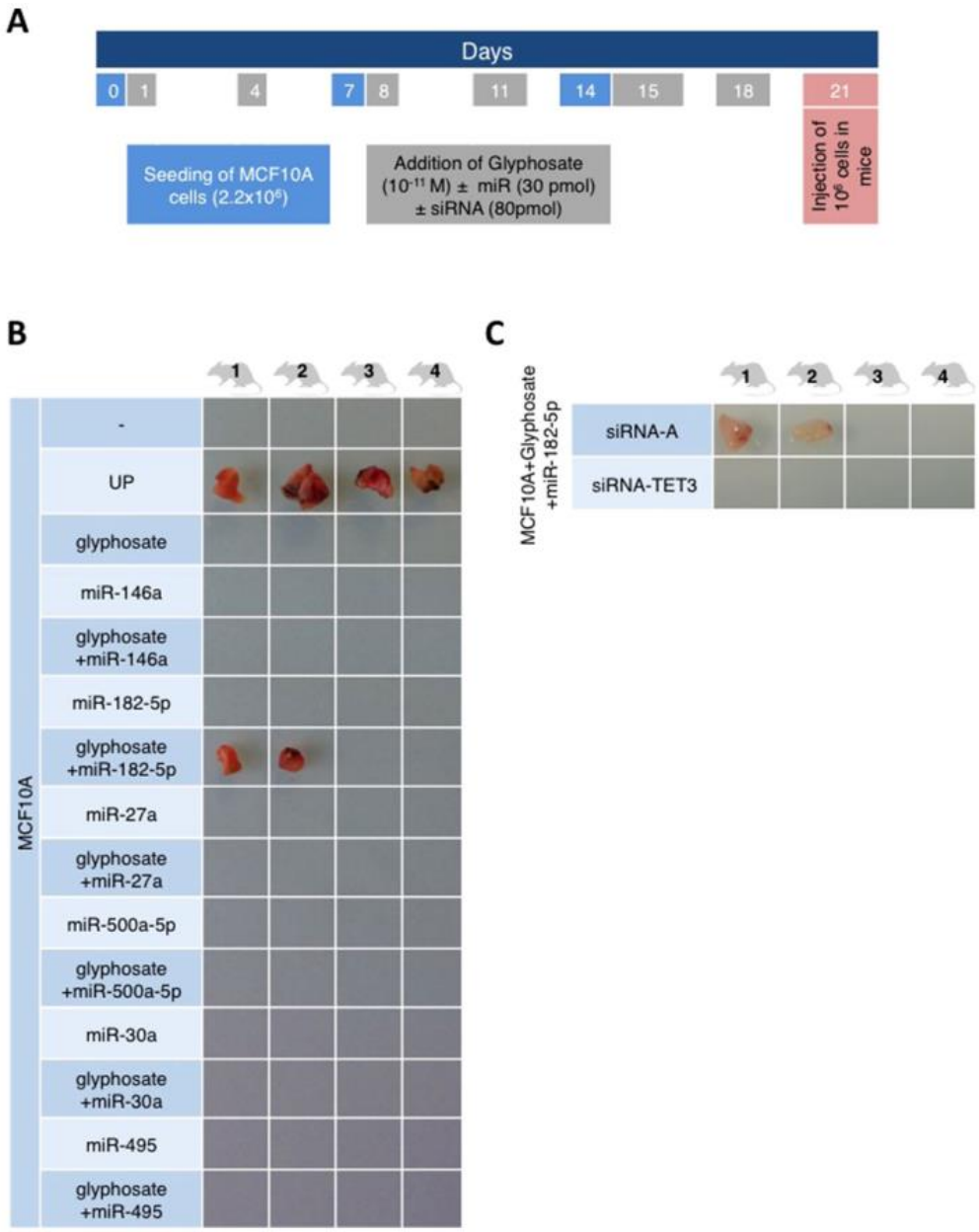


FIGURE 2 | The combination of glyphosate exposure and miR-182 overexpression is tumorigenic for MCF10A cells in a two-factor hit model. **(A)** The timetable illustrates the experiment design. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. **(B** and **C)** Four mice were injected per condition. miRCury LNA miR mimics and siRNA for TET3 were used to overexpress miRs or siRNA in MCF10A cells. Mice were euthanized 21 days after the injection of cells, and the tumors were resected. The pictures show the resected tumors.

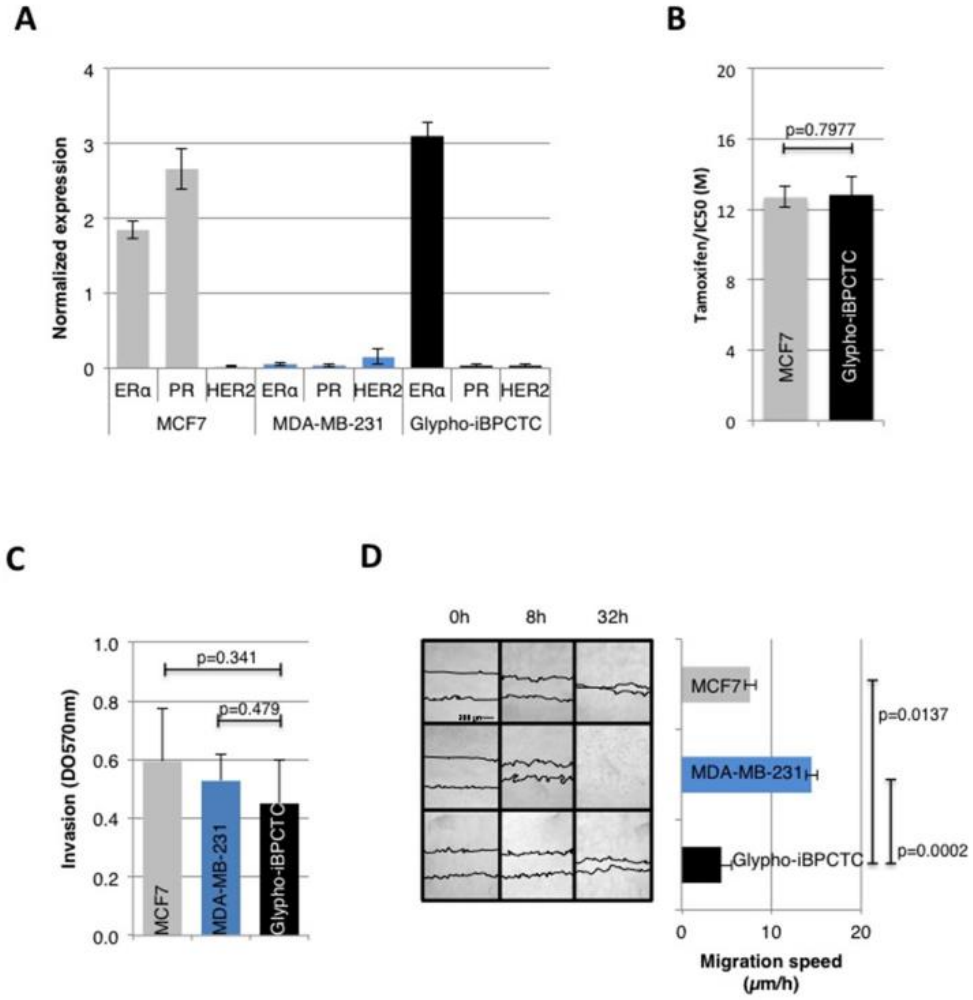


FIGURE 3 | Primary cells from glyphosate-induced breast tumor display characteristics of malignant cells. **(A)** The expression levels of ERα, PR, and HER2 were estimated in MCF7 cells, MDA-MB-231 cells, and Glypho-iBPCTC primary cells using In-Cell ELISA. Normalization to Janus Green staining intensity was performed to account for differences in cell seeding density. The bar graph displays the average ± standard deviation values of three independent experiments. **(B)** Bar graph of the viability of MCF-7 and Glypho-iBPCTC cells treated with increasing doses of tamoxifen (0, 2, 4, 6, 8, 10, 16, 19, 22 µM). Viability was measured by an MTT test, and the results represent the average ± standard deviation values of six independent experiments. The IC50 for each cell type was calculated using the IC50 Calculator (ATT Bioquest). **(C)** Bar graph showing the invasion capacity of MCF-7, MDA-MB-231, and Glypho-iBPCTC cells measured by optical density (absorbance at 570 nm). n = 3. **(D)** Confluent cultures of MCF-7, MDA-MB-231, and Glypho-iBPCTC cells were subjected to the wound healing test. The average migration speed was obtained by calculating the ratio distance/time between each acquisition time. Left: Pictures were acquired immediately after seeding (0 h) and after 8 and 32 h of culture. The bar graph represents the average ± standard deviation values of three independent experiments.

DMOG, a TET Inhibitor, Prevents Tumor Formation in Glyphosate-Challenged Cells; Some of the nutraceuticals/alicaments currently available target epigenetic pathways involved in normal homeostasis, notably those controlling DNA methylation. Like established epigenetic drugs, these sources of epigenetic modifiers offer great potentials to help determine the epigenetic path targeted by environmental factors and possibly revert the risk of tumorigenesis. MCF10A cells were transfected with miR-182-5p and exposed to 10-11 M of glyphosate ($MCF10A^{glyphosate/miR\text{-}182\text{-}5p}$) every 3 to 4 days over a 21-day period. They were also simultaneously treated with 40 µg/ml folate, a promoter of DNA methylation, or with 250 µM ascorbic acid, an activator of TET, 24 h after every glyphosate +/-miR treatment (Figure 4A). $MCF10A^{glyphosate/miR\text{-}182\text{-}5p}$ cells were also treated in a similar manner with two therapeutic agents, an anti-miR-182-5p (50 nM) and dimethyloxallyl glycine (DMOG, 1 mM), a compound that blocks TET enzymatic activity (Figure 4A). For all of these conditions, the global level of DNA methylation and tumor incidence compared to untreated MCF10Aglyphosate/miR-182-5p cells (control) at the end of the 21-day treatment sequence was measured. As expected, folate and DMOG prevented glyphosate-induced DNA demethylation, whereas ascorbic acid further reduced DNA methylation in MCF10Aglyphosate/miR-182-5p cells, as shown by the level of 5mC (Figure 4B).

Treatment with anti-miR-182-5p did not modify significantly the level of 5mC compared to control. Both folate and DMOG treatments were confirmed to indeed induce hypermethylation in several cell lines. Of the two hypermethylating agents, DMOG and folate, only DMOG prevented tumor formation; there was no difference between folate and control treatments (50 % of the mice displayed tumors). Ascorbic acid and glyphosate acting synergistically on DNA hypomethylation led to a 50 % increase in tumor incidence. In contrast, although without an obvious impact on glyphosate-induced DNA hypomethylation, anti-miR-182-5p was able to prevent tumor formation (Figure 4C). These results confirm that both DNA demethylation and miR-182-5p are necessary for tumor onset. Importantly, the extent of DNA demethylation appears to set a threshold for tumor onset (i.e. the more hypomethylated, the higher the risk for tumor development).

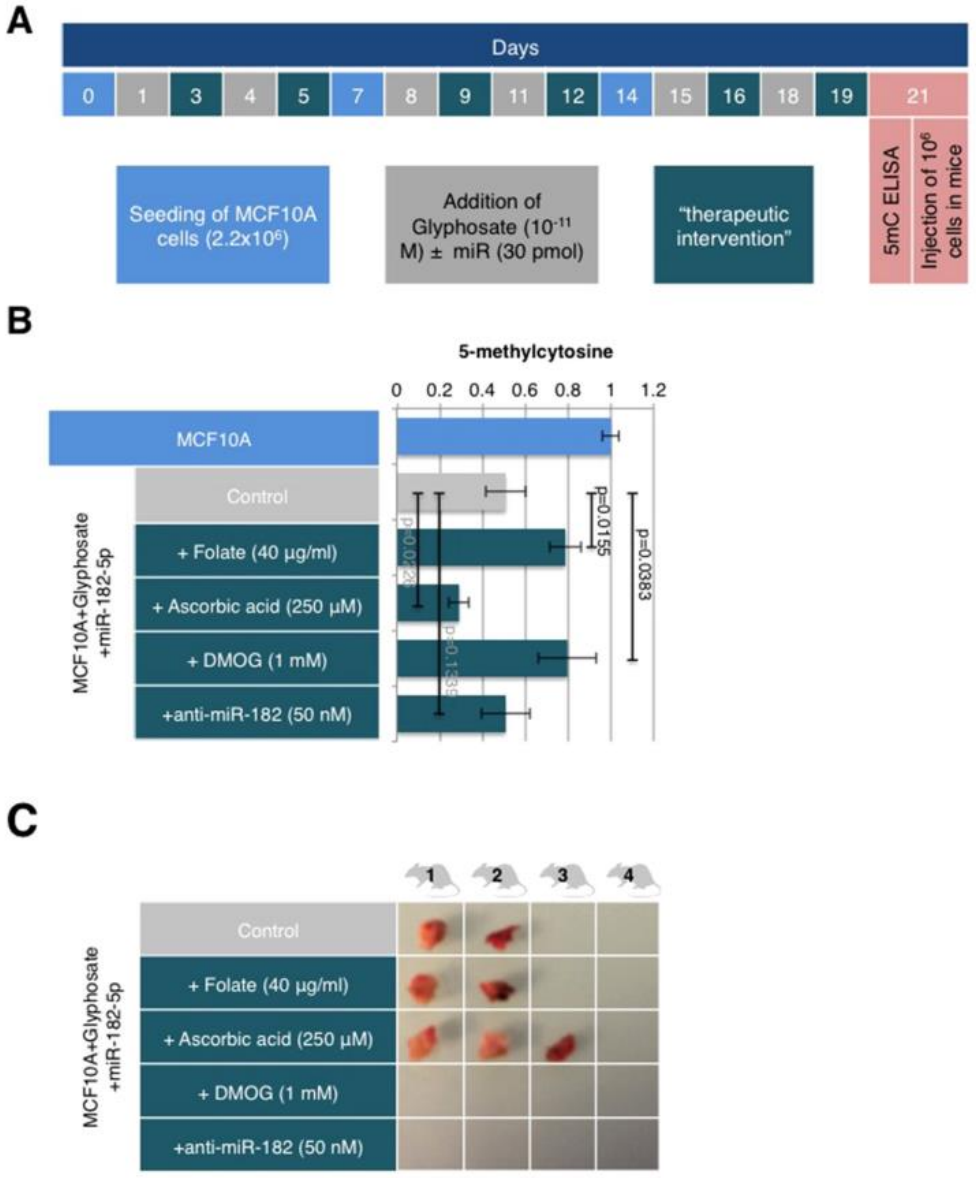


FIGURE 4 | DMOG and anti-miR-182 prevent tumor onset but differentially impact 5-meC level. **(A)** The timetable illustrating the experiment design. Explanations for color-coded days are located in corresponding color rectangles underneath the timeline. Therapeutic interventions on MCF10A cells treated with glyphosate and miR as indicated were performed on days 3, 5, 9, 12, 16, and 19 with folate (40 µg/ml), ascorbic acid (250 µM), DMOG (1 mM), or anti-miR-182 (50 nM). **(B)** MCF10A cells were treated as shown in schedule A. DNA was extracted at day 21 and used in 5mC ELISA. The bar graph illustrates the levels of 5mC for the different conditions. **(C)** Mice were injected with the cells following the treatment schedule A and euthanized 21 days later. Shown are pictures of the resected tumors.

Glyphosate Exposure Induces Sustained TET3-Mediated Gene Demethylation; The hypomethylation induced by glyphosate treatment is sufficient for tumor onset when using a two-factor hit model with

induced overexpression of miR-182-5p. Therefore, the possibility that an epimark of hypomethylation might be imprinted in the DNA was investigated. It was postulated that the putative epimark induced by glyphosate might be the hypomethylation of TET3-targeted genes because TET3 mediates glyphosate-induced DNA hypomethylation. The chromatin immunoprecipitation (ChIP) atlas database identifies MTRNR2L2, COL23A1, MSH3, DHFR, and DUX4 as the most frequently present in TET3-ChIP hits. According to this predictive finding, ChIP experiments using anti-TET3 antibody were performed for chromatin obtained from MCF10A cells treated or not with glyphosate for 21 days, such as described in Figure 1A. Interestingly, only MTRNRL2 and DUX4 genes were immunoprecipitated by TET3 in MCF10A cells treated with glyphosate. COL23A1, MSH3, and DHFR genes were not immunoprecipitated in both untreated and treated MCF10A cells. Thus, the prediction made by the ChIP atlas database was validated for MTRNRL2 and DUX4 genes and not for the COL23A1, MSH3, and DHFR genes, suggesting a context-dependent accessibility for this set of TET3-controled genes. Accordingly, quantitative methylation-sensitive restriction enzyme (qMSRE) revealed that MTRNRL2 and DUX4 genes were strongly methylated in control cells and became hypomethylated in MCF10A cells exposed to glyphosate (Figure 5A). The involvement of TET3 in the glyphosate-induced hypomethylation of DUX4 and MTRNR2L2 was confirmed by the abrogation with siRNA-TET3 of the glyphosate-induced hypomethylation of these genes (Figure 5B). Preliminary investigation of available breast tissue from breast cancer-free women confirmed the demethylation of DUX4 and MTRNR2L2 in a woman showing glyphosate exposure based on urinary test. However, the methylation status of the five genes immunoprecipitated by TET3, MTRNR2L2, DUX4COL23A1, MSH3, and DHFR, should be kept in consideration in the future because a woman with low glyphosate exposure displayed methylation on the five genes, hence suggesting that an epimark should consider the methylation status of all these genes in future investigations (Supplementary Figure S5). The stability of epigenetic changes is an important factor for long-term risk determination. MCF10A cells were exposed to glyphosate for 21 days (as previously described; Figure 1A) and then cultured without glyphosate for 1 and 6 weeks. The DUX4 and MTRNRL2 hypomethylations remained stable, as shown by qMSRE, even after exposure to glyphosate has seized (Figure 5C). bc-GenExMiner and KM plotter indicated that a high expression of DUX4 is associated with a poor prognosis, suggesting that genes controlled by TET3 might deserve additional scrutiny in breast cancer pathogenesis.

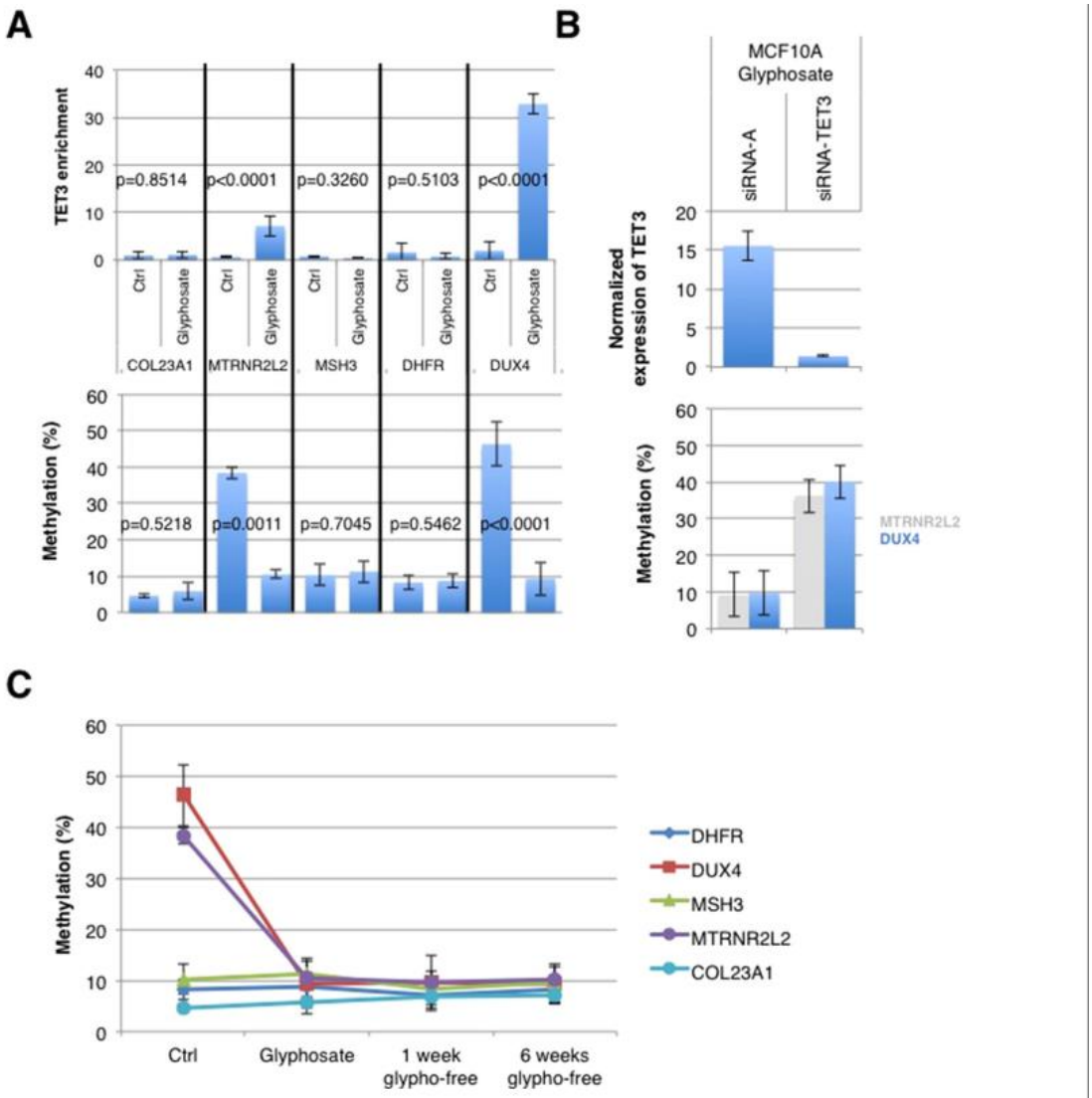


FIGURE 5 | Glyphosate-induced TET3-mediated demethylation affects *MTRNR2L2* and *DUX4* genes. **(A)** MCF10A cells were treated with glyphosate for 21 days as in the schedule shown in **Figure 2**. The graphs illustrate TET3 enrichment (top) following chromatin immunoprecipitation (ChIP) and the methylation level measured by qMSRE (bottom) of five genes defined by the ChIP atlas as being TET3-targeted genes. **(B)** MCF10A cells were treated with glyphosate for 21 days (according to the timetable of **Figure 2**), with siRNA added concomitantly to glyphosate. Bar graph (top) of TET3 expression measured with In-Cell ELISA after treatment with siRNA-TET3 (sc94636) or control siRNA-A (sc94636). Normalization to Janus Green staining intensity was performed to account for differences in cell seeding density. Bar graph (bottom) of methylation levels of *DUX4* and *MTRNR2L2* genes as measured by qMSRE. **(C)** MCF10A cells were treated with glyphosate for 21 days (glyphosate) according to the schedule shown in **Figure 1** and then cultured in glyphosate-free medium for another 1 (1 week glypho-free) or 6 (6 weeks glypho-free) weeks. Shown is the graph of the methylation level of five TET3-dependent genes. "Ctrl" represents MCF10A cells without glyphosate exposure.

Discussion

The impact of glyphosate on human health has been analysed and discussed for several years now. Recently, glyphosate exposure was correlated with shortened gestational lengths, and the level of glyphosate excretion was associated with steatohepatitis and advanced liver fibrosis in patients with fatty liver disease. However, the multiple research studies that investigated the tumorigenic effect of glyphosate as the sole risk factor had not led to convincing evidence of its implication.

It is assumed that only 5–10 % of cancers are directly caused by inherited genetic abnormalities. The remaining 90 % of cancers are linked to environmental factors that directly or indirectly affect DNA, possibly triggering genetic defects or aberrations in the reading and/or expression of DNA. Environmental and lifestyle factors are pleiotropic and include diet, tobacco, infections, obesity, alcohol, radiation, stress, physical activity, exposure to heavy metals and other pollutants, such as glyphosate. This study is reporting that glyphosate exposure is not oncogenic by itself, but it acts as an oncogenic hit factor that, combined with another oncogenic hit, promotes the development of mammary tumors.

At the molecular level, these findings demonstrate that glyphosate exposure can predispose breast cells to tumorigenesis *via* epigenetic reprogramming occurring *via* TET3-mediated global and local DNA hypomethylation.

This study and others have identified that global DNA hypomethylation promoting tumorigenesis may be caused by a deficiency of the DNMT1/PCNA/UHRF1 complex or of DNMT1 expression as shown in astrocytes, pulmonary fibroblasts, mesothelial cells, and breast cells. This study shows that glyphosate-mediated DNA hypomethylation is associated with TET3 overexpression instead of the DNMT1 pathway. The lower degree of DNA hypomethylation reached *via* the glyphosate TET3 path compared to that reached *via* UP peptide-DNMT1 path that is capable of inducing tumor onset suggests that a great intensity of global DNA hypomethylation could act as an oncogenic event, while a moderate intensity of global DNA hypomethylation might be considered a predisposing factor to cancer. The fact that active DNA demethylation orchestrated by TET can occur in resting (non-dividing) cells representing the majority of breast cells (in contrast to DNMT activity that requires cell proliferation) confers to TET-mediated mechanism a potentially higher degree of danger for cancer development.

The implication of TET proteins in breast cancer growth and metastasis has been strongly documented, and the level of hypomethylation of triple-negative breast cancer has been associated with TET1 DNA demethylase activity. In the latter article, it is proposed but not shown that TET1 might act as an oncogene by leading to aberrant hypomethylation. These findings demonstrate that the hypothesis of an involvement of TET-mediated DNA hypomethylation in cancer onset was correct. Notably, siRNA-TET3 abolished the presence of glyphosate-induced global and local DUX4 and MTRNR2L2 hypomethylation, as well as tumorigenesis. The data from this study feed the ongoing debate regarding whether TET3 exerts an oncogenic role or a tumor suppressor role. For the latter role, TET3 might act by inhibiting epithelial-to-mesenchymal transition in ovarian and melanoma cancers. But the current analysis with KM plotter database revealed a potentially unfavorable outcome for breast cancers when TET3 is overexpressed.

This work shows that two epigenetic events (global DNA hypomethylation and overexpression of a miR) cooperate to promote breast cancer. Other epigenetic events described to be involved in breast cancer development include the reduction of H3K9 acetylation *via* TIP60 downregulation that promotes ER-negative tumors. Histone acetyltransferase p300 activity and BIM1- mediated histone H2A ubiquitination that remodel chromatin are also two epigenetic events described as promoters for the development of aggressive breast tumors. A body of literature reports that miRs also play a crucial role in mammary tumorigenesis. In addition to oncogenic miRs, there are also miRs acting as tumor suppressors. For example, loss of miR-10b delays oncogene-induced mammary tumorigenesis overexpression of miR-489 inhibits HER2/neu-induced mammary tumorigenesis. Since the expression of miR depends on epigenetic control, it seems that either an extensive global hypomethylation of DNA (like with UP peptide) or a less extensive global hypomethylation associated with local epigenetic alterations affecting a miR might lead to tumor onset. The mechanisms associated with specific targeting of miR expression remain to be understood.

Breast cancer susceptibility has been statistically linked to epigenetic age acceleration and CpG island methylation. An important question is whether exposure to pollutants that are detrimental to epigenetic homeostasis might replace or synergize with age-related epigenetic changes and thus lead to the increase in earlier onset of breast cancer that is now documented. This possibility is further supported by our preliminary observation that the luminal B subtype of tumor (ER+/PR-/HER2-) triggered by glyphosate exposure combined with miR-182-5p overexpression is associated with poorer outcomes than the frequent ER+/PR+/HER2-luminal A type of tumor. Indeed, luminal B type of tumors have been found to be most common in young patients. This phenotype obtained from one tumor produced in mice will have to be confirmed with additional means; in any case, epigenetic markers of risk would be a prime resource to help curve the incidence. There exist already DNA methylation markers that add to the prediction of tertiary and secondary outcomes over and beyond standard clinical measures.

In the MCF10A model, glyphosate-induced DNA hypomethylation can be detected *via* the methylation level of only two of the five genes predicted to be controlled by TET3, MTRNR2L2 and DUX4 genes. Even if several other factors than glyphosate-induced TET3-mediated DNA hypomethylation (such as chromatin structure, other epimark, etc.) can govern the methylation status of the five genes, MTRNR2L2, DUX4, COL23A1, MSH3, and DHFR, this preliminary data with human samples support

the idea that the study of the methylation status of these five genes might be important to obtain a marker of risk based on a MethylGlypho score. The current study is pursuing this direction of research by detecting and analyzing this 5-gene TET3-dependent epimark in blood samples. Possibly, glyphosate induced methylome reprogramming might be used for the detection of an increased risk for breast cancer in women living in an environment conducive to this type of pollution.
Due to their concomitant expression during tumorigenesis associated with glyphosate-induced DNA hypomethylation, DUX4 and MTRNR2L2 may appear as players in this process instead of only be considered potential biomarkers. Results with KM plotter and bc-GenExMiner indicate that DUX4 level is negatively associated with breast cancer prognosis. No data seems available on MTRN2L2 in these databases. Based on the literature, DUX4 could act as an oncogene in various sarcomas and hematological malignancies, while information could not be found in the literature revealing a putative oncogenic role for MTRNR2L2. These TET3-controlled genes are worth further investigation to establish their causal effect in mammary tumorigenesis in future work.
Knowing the epigenetic pathway involved in glyphosate-mediated risk increase might lead to prevention strategies to follow detection of the epigenetic risk. The current findings suggest that TET-specific inhibitor DMOG might be a plausible therapeutic intervention since it gave a satisfactory response on both DNA methylation and tumor incidence. It would act by limiting TET3-mediated global DNA hypomethylation. In contrast, global remethylation of DNA by folate that has been considered for possible preventive effect is insufficient to prevent tumor incidence in the case of glyphosate exposure. Another interesting direction would be to limit the intake of ascorbic acid since it not only further reduced DNA methylation but also increased tumor incidence in mice. The epigenetic pathway leading to DNA hypomethylation is an important aspect to consider for further translational work on breast cancer risk.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to investigate DNA hypomethylation in MCF10A cells, tumorigenic response for MCF10A Cells in a two-factor hit model, prevention of tumor formation in glyphosate-challenged cells, and TET3-Mediated Gene Demethylation following glyphosate exposure. This study was conducted *in vitro* using only one level of glyphosate. Glyphosate was not correlated to environmental exposures. In the *in vivo* portion of the study, a sufficient number of animals were not used to determine a carcinogenic response for statistical analysis. While this study is acceptable as supplemental information on the *in vitro* effects of glyphosate, it is not appropriate for endpoint derivation in human health risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was insufficiently characterized and only one and extremely low concentration of glyphosate was used.

Reliability criteria for *in vitro* toxicology studies made by applicant

Publication : Duforestel *et al*., 2019.	**Criteria met? Y/N/ ?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y ?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity was not reported, source : Santa-Cruz,

		France.
Only glyphosate acid or one of its salts is the tested substance	Y	
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Non-neoplastic breast epithelial MCF10A cells.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	NA	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	One test concentration at 10^{-11}M, 10^{-5} µM (extremely low concentration) applied every 3 to 4 days over 21 days.
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Not possible with one concentration
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was insufficiently characterized and only one and extremely low concentration of glyphosate was used.		

Assessment and conclusion by RMS :
The study gives a good description of the study methods applied and the results obtained. The *in vitro* portion of the study is considered to be reliable without restrictions (Klimisch Score 1). The only limitations noted was that the purity of the test substance was not provided.

The applicants considered that the *in vivo* portion of the study was not fully reliable due to a low number of animals. In the *in vivo* portion of the study only four animals were used with the argumentation that the authors anticipated to detect highly frequent tumorigenic events. The low number of animals does indeed not allow for a statistical analysis. Nonetheless, the study still provides evidence that glyphosate can promote tumorigenesis in mice injected with MCF10A cells transfected with miR-182-5P. No effect was observed on non-transfected MCF10A cells nor in cells transfected with five other miRs transfected cells. In the study the cells were exposed to glyphosate *in vitro* after which infection into mice occurred. Due to the nature of this study design it is difficult to relate the exposure levels to *in vivo* situations. Therefore, while the study provides relevant information on *in vitro* effects of glyphosate it cannot be directly correlated to an adverse *in vivo* outcome.

In contrast to the applicant, the RMS does not consider the fact that only one dose was tested to be a limitation as the goal of the study was not to derive a dose-response relationship.

B.6.5.18.7. Supporting publications – Hao, 2019

Data point :	CA 5.5/032
Report author	Hao, Y. *et al*
Report year	2019
Report title	Roundup-Induced AMPK/mTOR-Mediated Autophagy in Human A549 Cells
Document No	doi.org/10.1021/acs.jafc.9b04679 E-ISSN : 1520-5118

Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previously submited	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable with restrictions

Full summary of the study according to OECD format

Glyphosate-based herbicide (GBH) is one of the most widely used pesticides based on a 5-enolpyruvylshikimate-3-phosphate synthase target, which does not exist in vertebrates. Here, the autophagic effects of the most famous commercial GBH Roundup (RDP) on human A549 cells *in vitro* has been studied. Intracellular biochemical assay indicated opening of mitochondrial permeability transition pore, LC3-II conversion, up-regulation of beclin-1, down-regulation of p62, and the changes in the phosphorylation of AMPK and mTOR induced by RDP in A549 cells. Further experimental results indicated that all the effects induced by RDP were related to its adjuvant polyethoxylated tallow amine, not its herbicidal active substance glyphosate isopropylamine salt. All these results showed that RDP has the ability to induce AMPK/mTOR-mediated cell autophagy in human A549 cells.

Materials and methods

Chemicals - Glyphosate isopropylamine salt (GP, ≥ 95 %) was obtained from Weihai Hanfu Biochemical Pharmaceutical Co., Ltd., Weihai, China. Polyethoxylated tallow amine (POEA) was obtained from AkzoNobel (Amsterdam, Netherlands). Roundup (RDP) containing 41% GP and 14.5% POEA was purchased from Monsanto (St. Louis, Missouri, USA).

Cell culture - DMEM supplemented with 1 % antibiotics (streptomycin and penicillin) and 10 % fetal bovine serum was used to culture human alveolar carcinoma A549 cells (ATCC, CCL-185). The cells were placed in an incubator at 37 °C with a humidified atmosphere of 5 % CO_2.

Cell viability - The MTT assay was performed to evaluate the cytotoxicity of glyphosate in A549 cells. 1.0×10^5 cells in 100 μL of fresh DMEM medium were plated in 96-well plates. After incubation for 24 hours, medium was replaced with fresh medium containing glyphosate at 100 μg/mL. Fresh medium served as the control. Three replicates were used per test group. After incubation for 2 hours, the MTT reagent (20 μL/well, 5 mg/mL) was added to form formazan crystals during incubation for 4 hours at 37 °C . The medium was removed and the formazan crystals were dissolved in DMSO (150 μL/well). Optical density (OD) was measured with a microplate reader at 570 nm. Percent cell viability inhibition was calculated as follows: $cell\ inhibition(\%) = (OD_{control} - OD_{treatment})/OD_{control} \times 100$. SPSS version 17.0 was used to calculate IC_{50} values.

Monodansylcadaverine (MDC) staining – MDC staining was used to mark autophagic vacuoles. A549 cells (1×10^5 cells/well) were grown in 24-well plates. The cells were exposed to glyphosate at 100 μg/mL for 2 hours. Fresh medium served as the control. After rinsing twice with PBS at pH 7.4, the cells were incubated with 10 μM MDC in the dark for 20 minutes at 37 °C . After washing with PBS at pH 7.4, the treated cells were photographed and analyzed by fluorescence microscopy at 488 nm at a magnification of 200×. Three photos were taken per group and analyzed to determine the number of autophagosomes. The effect of POEA (35 μg/ml), GP (100 μg/ml) and RDP (100 μg/ml) on A549 cells were tested by the same method.

Visualization of double-membrane autophagosomes - Visualization of the ultrastructure of A549 cells was performed by transmission electron microscopy (TEM). A549 cells were harvested after being treated for 2 hours with glyphosate at 100 µg/mL. Fresh medium served as the control. The cells were then rinsed twice with PBS at pH 7.4 and placed in a 2.5 % glutaraldehyde solution and kept overnight at 4 °C . Subsequently, the cells were post-fixed in 1 % osmium tetroxide (OsO_4) after rinsing with PBS at pH 7.4. The cells were then dehydrated with an ascending series of ethanol solutions and embedded with Epon 812. Ultrathin sections were stained with 2 % uranyl acetate and lead citrate solutions. The images were recorded using a JEOL JEM-2100 transmission electron microscope.

Autophagic flux - Ad-mCherry-GFP-LC3B is an adenovirus that expresses the mCherry-GFP-LC3B fusion protein, which is used to analyze autophagic flux after infection of cells. A549 cells were grown in 3 cm glass bottom cell culture dishes and infected with adenovirus (40 MOI) for 24 hours. After incubation for another 24 hours, the cells were exposed to GP at 100 µg/mL, POEA at 35 µg/ml and RDP at 100 µg/ml and incubated for 2 hours and then rinsed twice with PBS at pH 7.4. Fresh medium served as the control. The expression of mCherry and green fluorescent protein (GFP) was visualized and analyzed by a Nikon confocal microscope at 488 and 561 nm at a magnification of 100× by taking three photos per group. Diffuse yellow fluorescence indicates no occurrence of autophagy whereas yellow spots indicate autophagy. Autophagic flux was evaluated by the accumulation of mCherry-GFP-LC3B on the autophagosome membrane.

Colocalization of mitochondria and lysosomes - MitoTracker Green was used for mitochondria-specific fluorescent staining of live cells. LysoTracker Red was used for lysosomal-specific fluorescent staining of live cells. The two probes were used for mitochondrial and lysosomal colocalization imaging. A549 cells were treated with glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After treatment and rinsing, the cells were incubated with MitoTracker Green (0.5 µM) and LysoTracker Red (0.5 µM) for 30 minutes. Afterwards, the cells were photographed and analyzed by fluorescence microscopy at 488 and 561 nm at a magnification of 200×. Three photos were taken per group and analyzed. The effect of POEA (35 µg/ml), GP (100 µg/ml) and RDP (100 µg/ml) on A549 cells were tested by the same method. The counterstaining cells were photographed by a Nikon confocal microscope at a magnification of 100× and emission recorded at 488 nm for MitoTracker Green and 561 nm for LysoTracker Red. The related plugins (JACoP, colocalization threshold) of ImageJ v1.8.0 software were used to obtain a relative coefficient of colocalization between Mito and Lyso.

Opening of mitochondrial permeability transition pore (mPTP) - Opening of mPTP was measured by using calcein and $CoCl_2$. A549 cells were grown in 12-well plates and treated with glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After rinsing twice with PBS at pH 7.4, the cells were incubated with 1 mM calcein in the dark for 20 minutes at 37 °C and then exposed to 1 mM $CoCl_2$ for 30 minutes. The control group was not incubated with $CoCl_2$. After rinsing with PBS at pH 7.4, the treated cells were analyzed by fluorescence microscopy at 488 nm at a magnification of 200× by taking three photos per group. The effect of POEA (35 µg/ml), GP (100 µg/ml) and RDP (100 µg/ml) on A549 cells were tested by the same method.

Immunoblotting of proteins linked with autophagy - The relative protein expression levels of LC3, beclin-1, p62, p-AMPK, p-mTOR, and p-p70s6k in A549 cells were determined by immunoblot analysis to explore the underlying mechanisms of induced autophagy. A549 cells were treated with glyphosate at 100 µg/mL for 2 hours. Fresh medium served as the control. After treatment and rinsing the cells were lysed by a mixture of 50 µL immunoprecipitation assay lysis buffer with 0.5 µL protease inhibitor (100 mM). The total protein concentrations were determined using the Bicinchoninic Acid (BCA) Protein Assay Kit. Equal amounts of lysate proteins (50 µg) were separated by sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene fluoride membranes. The membranes with the proteins were blocked in Tris-buffered saline containing 5 % nonfat dried milk and 0.05 % Tween-20 for 2 hours. Blocked membranes were incubated with rabbit polyclonal antibodies for LC3, beclin-1,

p62, p-AMPK, p-mTOR, p-p70s6k, and β-actin overnight at 4 °C. After being washed three times in Tris-buffered saline containing 0.05 % Tween-20, membranes were incubated with anti-rabbit IgG secondary antibodies for 2 hours. The antibody-bound proteins were detected by Electro-Chemi-Luminescence kit and scanned by the chemiluminescent gel imaging system. The bands' grayscale values were quantified by ImageJ v1.8.0 software. The effect of POEA (35 µg/ml), GP (100 µg/ml) and RDP (100 µg/ml) on A549 cells were tested by the same method.

Adenosine triphosphate (ATP) content - The levels of intracellular ATP were determined by the ATP Assay Kit from Beyotime following the manufacturer's instructions. After treatment with glyphosate at 100 µg/mL or fresh medium as the control for 2 hours, A549 cells were lysed in the lysis buffer for 30 minutes at 4 °C . After centrifugation at 12,000 rpm for 15 minutes, a part of the supernatant was transferred into a light-protected 96-well plate for determination of the activity of ATP. The other part was used to determine the protein concentrations by the Pierce BCA Protein Assay Kit. The ATP concentrations (µmol/L) were converted to the protein concentrations (mg/L).

Statistical Analysis - Three separate replicates were performed for each assay. The statistical analysis process runs under SPSS version 17.0, statistical program (SPSS Inc). Data are presented as the means ± standard deviation (SD). Three independent experiments of MTT assay were analyzed by two-way ANOVA followed by Tukey post hoc testing. Different small alphabets indicate significant differences ($P \leq 0.05$). Others were subjected to one-way analysis of variance (ANOVA) followed by Dunnet's test for determining the differences with control ($^{*}P \leq 0.05$; $^{**}P \leq 0.01$).

Results

Viability - Inhibition of cell viability (as % of control) of A549 cells was 74.06 ± 4.90, 5.36 ± 0.82 and 68.81 ± 2.43 for POEA at 35 µg/ml, glyphosate at 100 µg/mL and Roundup at 100 µg/ml after 2 hours of treatment.

Monodansylcadaverine (MDC) staining - MDC staining in A549 cells treated with glyphosate at 100 µg/mL was not significantly different from the control. Roundup did induce MDC staining in A549 cells.

Visualization of double-membrane autophagosomes - The visualization of autophagosomes by TEM showed an homogeneous cytoplasm with normal mitochondria in A549 cells treated with glyphosate at 100 µg/mL. Roundup did induce mitophagy in A540 cells which appears to involve the AMPK/mTOR signaling pathway.

Autophagic flux - In the control and in the cells treated with glyphosate at 100 µg/mL, mCherry-GFP-LC3B is present in the form of diffuse yellow fluorescence which is indicative of the absence of autophagy. In the POEA- and Roundup-treated GFP fuorescence was decreased because of the acid environment with the mCherry-GLP-LC3B accumulating on the autophagosome membrane and appearing as yellow spots, which means the occurrence of autophagy induced by POEA and RDP.

Colocalization of mitochondria and lysosomes - The relative coefficient of colocalization between Mito and Lyso was 0.22 ± 0.01 for the control and 0.21 ± 0.02 for the cells treated with glyphosate at 100 µg/mL. The relative coefficients in the POAE and RDP-treated groups were 0.71 ± 0.07 and 0.59 ± 0.03, respectively.

Opening of the mitochondrial permeability transition pore (mPTP) - When compared with the $CoCl_2$-treated control group, no significant increase in fluorescence was noted in the cells treated with glyphosate at 100 µg/mL while and increase was observed for the POAE and RDP-treated groups which is indicative of the absence of mPTP opening.

Immunoblotting of proteins linked with autophagy - The effect of glyphosate, POEA and RDP on the

autophagy related proteins beclin-1, LC3-II/I, and p62 was studied by immunoblotting. When compared with the control no difference was found in the expression ratio of LC3-II/I as well as protein expression of beclin-1 in cells treated with glyphosate at 100 µg/mL. Immunoblotting was also used to examine the phosphorylation of mTOR, AMPK, and p70s6k. No difference in phosphorylation levels of mTOR, p70s6k, and AMPK could be demonstrated in cells treated with glyphosate at 100 µg/mL when compared to controls. These results indicate that glyphosate does not contribute to the activation of the AMPK/mTOR pathway. POEA and RDP did inhibit the levels of phosphorylated mTOR and phosphorylated p70s6k and the AMPK phosphorylation was promoted.

Adenosine triphosphate (ATP) content - When compared with the control, no difference was found in the levels of cellular ATP of cells treated with glyphosate at 100 µg/mL. Decreased levels of cellular ATP were noted in POEA and RDP treated A549 cells.

Conclusion

All commercial glyphosate formulations are more toxic than glyphosate so that the increased toxicity may be attributed to the adjuvants. The herbicidal active substance of Roundup is glyphosate isopropylamine salt and its main adjuvant is polyethoxylated tallow amine (POEA). To explore whether the herbicidal active substance or the adjuvant contributes Roundup's ability of inducing autophagy in human A549 cells, TEM, MDC, immunoblotting, and Ad-mCherry-GFP-LC3B analyses were performed on 4 concentrations of Roundup from 50 to 125 µg glyphosate acid eq./mL and one concentration of glyphosate (100 µg/mL) and POEA (35 µg/mL). This study revealed that the commercial formulation Roundup has the ability to cause autophagy in A549 cells *via* the AMPK/mTOR signaling pathway. Previous studies showed that compared with glyphosate, commercial glyphosate based herbicides are more toxic, and the toxic effect is highly correlated with the adjuvant POEA. This study indicates that the adjuvant POEA in Roundup contributes to its ability of inducing autophagy in A549 cells, and that the herbicidal active substance glyphosate has no contribution.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The effect of glyphosate, POEA and a herbicidal formulation containing POEA as a co-formulant on the AMPK/mTOR signaling pathway was studied in human alveolar carcinoma A549 cells *in vitro*. Only the results of exposure to glyphosate at 100 µg/mL are reported and discussed in this summary. The endpoints selected to study the effect of glyphosate on autophagy are inhibition of viability, monodansylcadaverine (MDC) staining to mark autophagic vacuoles, visualization of double-membrane autophagosomes by TEM, autophagic flux, colocalization of mitochondria and lysosomes, opening of the mitochondrial permeability transition pore (mPTP), expression of proteins involved in the AMPK/mTOR signaling pathway, and ATP content. No effect could be demonstrated of glyphosate on any of these endpoints indicating that glyphosate, in contrast to POEA and Roundup, does not contribute to the activation of the AMPK/mTOR signaling pathway and has thus no role in autophagy.

This publication is relevant for the risk assessment of glyphosate but reliable with restrictions because only one glyphosate concentration was tested and no positive controls were used.

Reliability criteria for *in vitro* toxicology studies

Publication: Hao, Xu *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	

Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of ≥95 %. Source: Weihai Hanfu Biochemical Pharmaceutical Co., Ltd.,China.
Only glyphosate acid or one of its salts is the tested substance	N	Also GBH (Monsanto, St. Louis, USA) and POEA tested.
AMPA is the tested substance	N	
Study		
Test system clearly and completely described	Y	Human alveolar carcinoma A549 cell line.
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Glyphosate only at one concentration tested: 100 µg/mL.
Cytotoxicity tests reported	Y	
Positive and negative controls	N	No positive control.
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Only one concentration for glyphosate.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is relevant for the risk assessment of glyphosate but reliable with restrictions because only one glyphosate concentration was tested and no positive controls were used.		

Assessment and conclusion by RMS:
The RMS agrees with the assessment that the study is reliable with restrictions but for slightly different reasons than the applicant. In general, the RMS does not consider the single dose level to be a limitation as the purpose of the study was not to derive a dose-response relationship. However, the dose levels of 100 µg/ml for glyphosate did not lead to a strong cytotoxic response (cell viability inhibition of only 5%) and it therefore seems that for glyphosate a higher concentration could have been tested although the aim of the study was to compare similar concentrations of Roundup, glyphosate and tallow amine separately.

B.6.5.18.8. Supporting publications - Pahwa, 2019

Data point:	CA 5.5/033
Report author	Pahwa, M. *et al.*
Report year	2019
Report title	Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project
Document No	doi:10.5271/sjweh.3830 E-ISSN: 1795-990X
Guidelines followed in study	None
Deviations from current test guideline	No

Previous evaluation	No, submitted for the purpose of the renewal
GLP/Officially recognised testing facilities	Not applicable for epidemiologic studies
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable with restrictions

Full summary of the study according to OECD format

In this paper a pooled reanalysis of the data from 2 published non-Hodgkin's lymphoma (NHL) case control studies was conducted: McDuffie *et al*. 2001 and DeRoos *et al*. 2003. The reanalysis sought to evaluate associations for glyphosate use and NHL overall and by histological sub-type. In addition, the pooled analysis implemented more extensive control of confounding factors than in the original publications and considered the impact of excluding pesticide information provided by next-of-kin or proxy respondents.

The OR for NHL overall for ever using glyphosate was 1.43 (95 % CI 1.11, 1.83). After adjustment for other pesticides, the OR was reduced to 1.13 (95 % CI 0.84, 1.51) with a statistically significant association for handling glyphosate >2 days/year (OR 1.75, 95% CI 1.02-2.94). In pesticide-adjusted sub-type analyses, the ordinal measure of lifetime-days was statistically significant (P=0.03) for SLL, and associations were elevated, but not statistically significant, for ever years or days/year of use. Handling glyphosate >2 days/year had an excess of DLBCL (OR 2.14, 95% CI 1.07–4.28; P-trend=0.2). However, as indicated by the study author consistent patterns of association across different metrics were not observed.

There was some limited evidence of an association between glyphosate use and NHL in this pooled analysis. Suggestive associations, especially for SLL, deserve additional attention.

Materials and methods

Study population and exposure assessment
Pahwa *et al*. pooled data from case control studies in the US and Canada. For NHL specifically, this study is essentially a reanalysis of the published studies by McDuffie *et al*. (2001) in Canada and DeRoos *et al*. (2003) in the US. Case identification in the US was through cancer registries and hospitals during the 1980s in four US states (Iowa/Minnesota, Kansas, and Nebraska) and between 1991 and 1994 in six Canadian provinces (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia). Methods for each study have been previously described. For this pooled analysis, the original histology codes were revisited and classified according to a single scheme [International Classification of Diseases for Oncology version 1 (ICD-O-1)].

Participants, or their proxies, provided information about demographic characteristics, pesticide use, agricultural exposures, and exposure to other known or suspected NHL risk factors, including lifestyle and medical and occupational history. Self-reported glyphosate use was examined using several exposure metrics: ever/never, duration (years used), frequency (days/year handled), and lifetime-days (number of years used multiplied by number of days/year handled). Categories were created for duration, frequency, and lifetime-days analyses based on the median of glyphosate used/handled among controls. Some participants had missing data for duration and frequency of glyphosate use despite reporting that they had ever used glyphosate. In duration and frequency analyses, values for missing data were assigned to cases and controls based on the median duration or frequency of reported glyphosate use among controls by state/province and 10-year age group (simple imputation) and were used for the main analyses. Ordinal analyses and associated trend tests were conducted to determine possible changes in association for increasing increments of every five years, five days/year, and ten lifetime-days of

glyphosate use.

Statistical analyses
Unconditional multiple logistic regression was performed using the LOGISTIC procedure of the SAS 9.4 statistical software package (SAS Institute, Cary, NC, USA) to calculate ORs and 95 % CIs for associations between glyphosate exposure metrics (ever/never, duration, frequency, lifetime-days, and as ordinal variables) and NHL overall and by histological sub-type [diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), small lymphocytic lymphoma (SLL), and other]. Initial logistic regression models (OR) contained the following variables: age [age at diagnosis (cases); age at interview or death (controls)], state/province, sex, lymphatic or hematopoietic cancer in a first-degree relative, response by a proxy, and use of any personal protective equipment (PPE). Pesticides that were correlated with glyphosate use in the pooled data and that had previously been associated with NHL based on the individual case-control studies, specifically 2,4-dichlorophenoxyacetic acid, dicamba and malathion, were included in the more fully adjusted logistic regression models (OR). The former models will be referred to as crude and the latter models as adjusted.

Trends for duration, frequency, and lifetime-days of glyphosate use and NHL ORs were assessed by the asymptotic Cochran-Armitage trend test. Subjects who never used glyphosate were the reference group for all analyses. There was a small proportion of subjects (N=175, 2.6 % of all participants) with missing age values. These were imputed using simple imputation based on state/province- and case/control-specific means of age rounded to the nearest whole number. Sensitivity analyses were conducted by excluding proxy respondents from the main analyses.

Ethics approval and consent to participate
Ethics approval for the pooled analysis was obtained from the University of Toronto Health Sciences Research Ethics Board (#25166) and an exemption was obtained from the US National Institutes of Health Office of Human Subjects Research (#11351). Investigators of individual studies received human subjects' approval from their institutions for each study prior to collection of data.

Results

Characteristics of NHL cases and controls
A total of 1690 NHL cases and 5131 controls was available for analysis – 69.6 % of the cases and 70.6 % of the controls were from the US studies. All NHL cases and controls, including those with proxy respondents, were included in analyses of ever/never glyphosate use. For assessments involving duration of use, 1520 cases and 4183 controls were included. For frequency and lifetime-days analyses, 898 cases and 2938 controls were included. The numbers of cases and controls available for the sensitivity analysis excluding proxy respondents were smaller (Figure B.6.5.18.8-1). Characteristics of NHL cases and controls, including histological sub-types, are presented in Table B.6.5.18.8-1.

Figure B.6.5.18.8-1. Subjects in main and proxy respondent analyses of glyphosate use and NHL in the North American Pooled Project (NAPP). * Duration (years) information was not collected in Kansas, ** Frequency (days/year) information was not collected in Iowa, Minnesota, and Kansas.

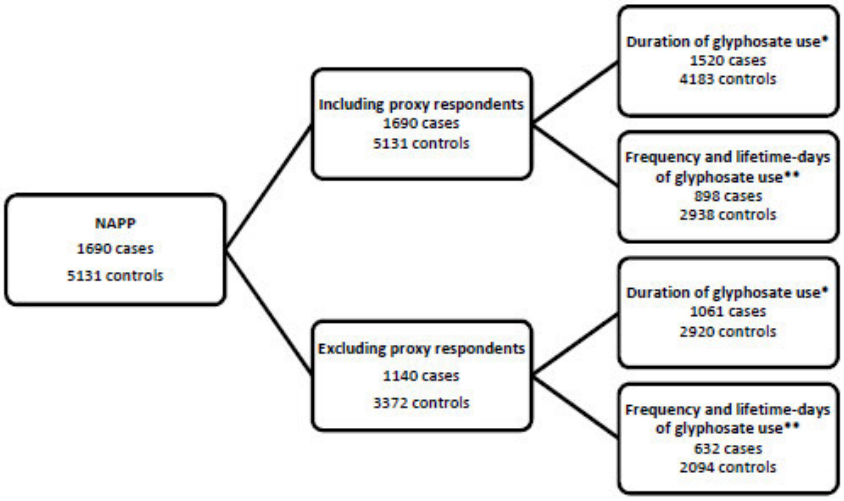


Table B.6.5.18.8-1: Characteristics of non-Hodgkin lymphoma (NHL) cases and controls in the North American Pooled Project (NAPP). [OR=odds ratio; CI=confidence interval].

Characteristics	Cases (N=1690)		Controls (N=5131)			
	N	%	N	%	OR [a]	95% CI
Histological sub-type						
Diffuse large B-cell lymphoma (DLBCL)	647	38				
Follicular lymphoma (FL)	468	28				
Small lymphocytic lymphoma (SLL)	171	10				
Other	404	24				
State/Province U.S						
Nebraska	385	22	1432	28		
Minnesota	329	19	642	13		
Iowa	293	17	603	12		
Kansas	170	11	948	18		
Canada						
Ontario	142	8	585	11		
British Columbia	126	7	230	4		
Quebec	117	7	291	6		
Alberta	65	4	196	4		
Manitoba	34	2	113	2		
Saskatchewan	29	2	91	2		
Age (years) [b]						
≥19–≤29	26	2	277	5		
≥30–≤39	97	6	445	9		
≥40–≤49	159	9	514	10		
≥50–≤59	288	17	726	14		
≥60–≤69	564	33	1264	25		
≥70–≤79	402	24	1189	23		
≥80–≤89	137	8	610	12		
≥90	17	1	106	2		
Sex						
Male	1506	89	4424	86	1.00	ref
Female	184	11	707	14	0.94	0.75–1.17
Respondent type						
Self	1140	67	3372	66	1.00	ref
Proxy	533	32	1692	33	1.03	0.90–1.17
Unknown/missing	17	1	67	1		
Lymphatic or hematopoietic cancer in a first-degree relative						
No	1493	88	4790	93	1.00	ref
Yes	139	8	202	4	2.13	1.69–2.67
Unknown/missing	58	3	139	3		
Ever diagnosed with selected medical conditions [c]						
No	1011	60	3346	65	1.00	ref
Yes	545	32	1389	27	1.12	0.92–1.37
Unknown/missing	134	8	396	8		
Ever used any type of personal protective equipment						
No	374	22	1127	22	1.00	ref
Yes	105	6	310	6	1.12	0.86–1.45
Unknown/missing	1211	72	3694	72		

[a] Adjusted for age and state/province.
[b] Cases - mean 62.72 (SD 13.78) years; Controls - mean 61.66 (SD 17.13) years.
[c] Ever diagnosed with ≥1 of the following select medical conditions: allergies (any, food, or drug), asthma, hay fever, infectious mononucleosis, rheumatoid arthritis, tuberculosis, or received chemotherapy or radiation therapy.

Glyphosate use and associations with NHL overall and by major histological sub-type

Overall, 113/1690 cases (7 %) and 244/5131 (5 %) controls reported having used glyphosate at any point in their lifetime. In crude analyses, there was a significant association between ever use of glyphosate and NHL overall (OR1.4, 95 % CI 1.1–1.8) that was attenuated appreciably when adjusted for ever use of the pesticides 2,4-D, dicamba, and malathion (OR1.1, 95 % CI 0.8–1.5) (Table B.6.5.18.8-2).[2] Adjusted ORs by for NHL subtypes were: 0.6 (95 % CI 0.4 – 1.2) for FL, 1.2 (95 % CI 0.8–1.9 for DLBCL, 1.8 (95 %CI 0.9 – 3.7) for SLL, and 1.5 (95 % CI 0.9 – 2.6) for other NHL subtypes.

Table B.6.5.18.8-2: Ever/never glyphosate use and association with non-Hodgkin lymphoma (NHL) overall and histological sub-types in the North American Pooled Project (OR = odds ratio; CI = confidence interval). Note: proxy respondents included

	Never-used glyphosate		Ever-used glyphosate				
	N	OR [a,b]	N	OR [a]	95% CI [a]	OR [b]	95% CI [b]
Controls	4887	1.00 (ref)	244				
NHL overall	1577	1.00 (ref)	113	1.43	1.11–1.83	1.13	0.84–1.51
Follicular lymphoma (FL)	440	1.00 (ref)	28	1.00	0.65–1.54	0.69	0.41–1.15
Diffuse large B-cell lymphoma (DLBCL)	602	1.00 (ref)	45	1.60	1.12–2.29	1.23	0.81–1.88
Small lymphocytic lymphoma (SLL)	156	1.00 (ref)	15	1.77	0.98–3.22	1.79	0.87–3.69
Other	379	1.00 (ref)	25	1.66	1.04–2.63	1.51	0.87–2.60

[a] Adjusted for age, sex, state/province, lymphatic or hematopoietic cancer in a first-degree relative, use of a proxy respondent, use of any personal protective equipment.

[b] Adjusted for age, sex, state/province, lymphatic or hematopoietic cancer in a first-degree relative, use of a proxy respondent, use of any personal protective equipment, use of 2,4-D, use of dicamba, and use of malathion.

When ORs for NHL and glyphosate use were examined by duration, there were lower ORs with longer use for NHL overall and for subtypes except SLL (see table B.6.5.18.8-3). Conversely, there were higher ORs for those who reported use for more than 2 days per year verses those who reported use ≤ 2 days per year. Analyses by lifetime days of use – the metric used in most studies – showed near null results for all subtypes in the higher lifetime days category, except for SLL. It bears noting that the analyses by duration included 90 % of cases and controls (Kansas subjects did not have the required data), while the analyses of days/year and lifetime days included only 53 % of subjects (cases and controls from Kansas, Iowa, and Minnesota did not have the required data and were excluded). As such, these latter analyses may not be representative of results for the entire pooled study population.

Table B.6.5.18.8-3: Adjusted Odds Ratios (95 % confidence intervals) by Various Glyphosate Exposure Metrics

Metric	NHL overall	FL	DLBCL	SLL	Other NHL
duration					
≤ 3.5 years	1.3 (0.9, 1.8)	0.7 (0.3, 1.3)	1.6 (0.97, 2.7)	1.4 (0.6, 3.7)	1.8 (0.95, 3.5)
> 3.5 years	0.9 (0.6, 1.4)	0.6 (0.3, 1.3)	0.9 (0.5, 1.7)	1.9 (0.8, 4.8)	1.1 (0.5, 2.5)
P-trend	0.9	0.1	0.7	0.1	0.4
days/year					
≤ 2	0.7 (0.5, 1.2)	0.5 (0.2, 1.2)	0.7 (0.4, 1.4)	1.3 (0.4, 4.3)	1.1 (0.5. 2.7)
> 2	1.7 (1.0, 2.9)	1.3 (0.6, 3.2)	2.1 (1.1, 4.3)	2.3 (0.6, 8.8)	1.6 (0.6, 4.5)
P-trend	0.2	0.9	0.2	0.2	0.4
lifetime days					
≤ 7	0.9 (0.5, 1.5)	0.6 (0.3, 1.6)	0.8 (0.4, 1.7)	1.0 (0.2, 4.8)	1.4 (0.6, 3.5)
> 7	1.1 (0.7, 1.8)	0.8 (0.3, 1.8)	1.1 (0.6, 2.2)	2.2 (0.7, 6.9)	1.3 (0.5, 3.3)
P-trend	0.9	0.4	0.9	0.2	0.5

[2] Adjusting for other pesticides appreciably changed the NHL OR for glyphosate. Therefore, it seems most appropriate to focus on the ORs from the adjusted analyses.

Sensitivity analyses excluding proxy respondents

A sensitivity analysis was performed by excluding cases and controls whose data were provided by proxy respondents. The overall pattern of OR estimates were generally similar to the main analyses. However, for SLL, ORs were slightly higher in the > 2 days/year subgroup OR 2.3 (95%-CI 0.6-8.8) with proxies compared to OR 2.6 (95%-CI 0.7-10.1)) without proxies. For other NHL subtypes, ORs were slightly to appreciably higher for ever use with proxies OR 1.5 (95%-CI 0.9-2.6) compared to OR without proxies of 1.0 (95%-CI 0.5-1.9); OR for > 3.5 years of use 1.1 (95%-CI 0.5-2.5) compared to OR without proxies 0.5 (95%-CI 0.2-1.6); and OR for > 7 lifetime days with proxies 1.3 (95%-CI 0.5-3.3) compared to OR without proxies of 1.1 (95%-CI 0.4-3.3).

Note RMS: the ORs without proxies cited above were reported in the supplementary information which is publicly available online.

Discussion & Conclusion

The objective of this study was to evaluate potential associations between glyphosate use and NHL in the NAPP, a pooled dataset that allowed for a more comprehensive analysis than previously possible in the individual studies. Results from this analysis provide some limited, but inconsistent, evidence for an association between NHL overall and ever reported use of glyphosate. For NHL overall, the OR for ever use of glyphosate and NHL was near null when adjusted for reported use of 2,4-D, dicamba, and malathion (OR1.1, 95 % CI 0.8, 1.5). Analyses by years of use and lifetime days of use showed near null results for NHL overall (OR 0.9 and 1.1, respectively), while the OR for > 2 days/year was elevated (OR 1.7, 95 % CI 1.0, 2.9). It bears noting, however, that the analyses by days per year (</> 2 days/year) and lifetime days of use (</> 7 lifetime days) included only 50 % of the pooled population – essentially the Canadian subjects and 1 of the 4 US case-control studies. It is uncertain, therefore, how representative these results are for the entire pooled population. The results of those analyses should be interpreted accordingly.

In analyses of NHL sub-types, there tended to be moderate positive associations between the various glyphosate exposure metrics and SLL. However, as there were only 15 SLL cases overall who ever used glyphosate and 14, 7, and 7 exposed SLL cases in the analyses by years of use, days per year, and lifetime days of use, respectively, the results were not statistically significant and the 95 % CIs were imprecise. For other cell types, there were moderate positive relationships for glyphosate use of more than 2 days per year, though ORs were near null for these cell types for the higher category of years of use and lifetime days of use.

Assessment and conclusion by applicant:

The main advantage of this pooled analysis compared with the previously published individual studies was to enable a more comprehensive analysis for glyphosate with regard to confounding factors and proxy respondents. In general, adjusting for use of 2,4-D, dicamba, and malathion reduced ORs for glyphosate. Analyses that excluded proxy respondents were generally similar to analyses that included them, though there were some instances, specifically for other NHL subtypes, where excluding proxies appreciably reduced the adjusted OR.

Left unaddressed in this pooled analysis is the intractable issue of case-recall bias in case control studies. Crump has shown in an analysis of all the case control studies that have reported ORs for glyphosate, including the studies in this pooled analysis, that results for all pesticides were markedly skewed toward positive associations (Crump K, Risk Analysis DOI: 10.1111/risa.13440). Crump noted particularly that the ORs for individual pesticides in the McDuffie *et al*. study (and 2 other studies not included in this pooled analysis) were nearly all greater than 1.0. He considered this evidence of case recall bias. Fundamentally, using self-reported exposure recollections from cases and controls violates the basic principle that data should be collected under equivalent circumstances

for the groups to be compared (viz., cases and controls). That is impossible when pesticide recall is likely to be affected by their grievous illness for cases and not for controls. Accordingly, while this pooled analysis is an advance in understanding confounding by other pesticides and in assessing the impact of reporting by proxies (except in analysis where 50 % of the subjects were excluded due to data limitations) in the 2 included studies, systematic error related to case recall bias remains an outstanding issue for interpreting the results for glyphosate.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because it concerns a pooled case control study which is subject to recall and selection bias. Notably, potential case-recall bias remains an unresolved issue in this pooled reanalysis.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Pahwa M. *et al.*, 2019	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	Pooled reanalysis of 2 previously published studies.
Appropriate study population to address potential glyphosate-related health outcomes	Uncertain	Populations had very limited glyphosate exposure frequency.
Exposure studied		
Exposure to formulations with glyphosate as a.s.	Yes	
Exposure to formulations with other a.s.	Yes	
Exposure to other farm exposures	Uncertain	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	No	Much less participation by controls: example: McDuffie study – participation 67 % for cases, 48 % for controls.
Information provided by proxy respondents	Yes, substantial	31 % for cases, 40 % for controls in DeRoos study; 21 % for cases, 15 % for controls McDuffie study (per Chang & Delzell 2016)
Adequate statistical analysis	Yes	More comprehensive than the original publications regarding confounding & proxy responses. Data for 47 % of subjects were missing for analyses

Publication: Pahwa M. *et al.,* 2019	**Criteria met? Y/N/?**	**Comments**
		by days of use per year and lifetime cumulative days of use.
Adequate consideration of personal confounding factors	Yes	Better than original studies.
Adequate consideration of potentially confounding exposures	Yes	Better than original studies
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Yes	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because illustrates bias toward positive findings for glyphosate in the original publications due to confounding and, in part, due to proxy responses. Recall bias unresolved. Missing data for 47 % of subjects for analyses by days/year of use and lifetime cumulative days of use hinders interpretation of related results.		

Assessment and conclusion by RMS:

The study concerns the pooled analysis of data from McDuffie et al, 2001 and DeRoos et al. 2003. The reliability of these studies are already assessed elsewhere (B.6.5.18.23 and B.6.5.18.15, respectively). As the reliability of these studies were considered 'reliable with restrictions' and as only some of the limitations have been addressed (see next paragraph) also this pooled analysis is considered 'reliable with restrictions'.

Some of the limitations that were noted in the McDuffie et al 2001 study have been addressed here. The main one being that the ORs that were calculated for number of days exposed and lifetime exposure in this pooled analysis were adjusted for confounders which was not the case in the McDuffie study.
Compared to the DeRoos et al. 2003 study it is noted that more cases and controls were included in the analysis from Pahwa, 2019 as subjects with missing pesticide data were not excluded from analysis. This may be one of the explanation between the difference in the outcome of the De Roos, 2003 study which found an association between ever use of glyphosate and NHL and this Pahwa, 2019 study.

B.6.5.18.9. Supporting publications – Wang, 2019

Data point:	CA 5.5/034
Report author	Wang, L. *et al.*
Report year	2019
Report title	Glyphosate induces benign monoclonal gammopathy and promotes multiple myeloma progression in mice
Document No	doi.org/10.1186/s13045-019-0767-9 E-ISSN: 1756-8722
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previously submitted	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Evaluation GRG : Yes/Reliable with restrictions Evaluation AGG : Reliable with restrictions due to low number of

	animals.

Full summary of the study according to OECD format

Glyphosate is the most widely used herbicide in the USA and worldwide. There has been considerable debate about its carcinogenicity. Epidemiological studies suggest that multiple myeloma (MM) and non-hodgkin lymphoma (NHL) have a positive and statistically significant association with glyphosate exposure. As a B cell genome mutator, activation-induced cytidine deaminase (AID) is a key pathogenic player in both MM and B cell NHL.
Vk*MYC is a mouse line with sporadic MYC activation in germinal center B cells and considered as the best available MM animal model. Vk*MYC mice and wild-type mice were treated with drinking water containing 1000 mg/L of glyphosate and examined animals after 72 weeks.
Vk*MYC mice under glyphosate exposure developed progressive hematological abnormalities and plasma cell neoplasms such as splenomegaly, anaemia, and high serum IgG. Moreover, glyphosate caused multiple organ dysfunction, including lytic bone lesions and renal damage in Vk*MYC mice. Glyphosate-treated wild-type mice developed benign monoclonal gammopathy with increased serum IgG, anaemia, and plasma cell presence in the spleen and bone marrow. Finally, glyphosate upregulated AID in the spleen and bone marrow of both wild-type and Vk*MYC mice.
These data support glyphosate as an environmental risk factor for MM and potentially NHL and implicate a mechanism underlying the B cell-specificity of glyphosate-induced carcinogenesis observed epidemiologically.

Materials and methods

Mouse model and treatments; All chronic and acute animal experiments were performed in accordance with NIH guidelines and under protocols approved by the Cleveland Clinic Institutional Animal Care and Use Committee. Wild-type (WT) C57Bl/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Vk*MYC mice in the C57Bl/6 genetic background were obtained from Dr. Leif Bergsagel (Mayo Clinic, Scottsdale, AZ). Vk*MYC and WT mice were intercrossed to obtain WT and Vk*MYC littermates. Sex-matched WT and Vk*MYC mice (8 weeks old) were assigned to treatment or control groups based on body weight. For chronic study of glyphosate effects, treatment groups were provided 1.0 g/L glyphosate (Sigma-Aldrich, St. Louis, MO) in their drinking water for 72 weeks. Regular drinking water was provided for the control groups (Fig. 1a). Every 6 weeks, blood was collected from the tail vein of mice, and the serum IgG level was measured. At week 72, the remaining 3 surviving Vk*MYC mice reached humane endpoints. These 3 treated Vk*MYC mice were used for M-spike detection and pathologic analyses, along with mice from other groups. Other Vk*MYC mice that were sacrificed before week 72 were analysed for total serum IgG levels, complete blood cell count, and total serum creatinine. For comparison, mice from other groups were euthanized at week 72 and their tissues and blood analyzed. For acute treatment, 8-week old mice (n = 5 per group) were given 0, 1.0, 5.0, 10.0, or 30.0 g/L of glyphosate for 7 days before sacrifice. The same variables were analyzed in the acute study.

Blood and post-mortem assays; Whole-blood complete blood count (CBC), IgG enzymelinked immunosorbent assay (ELISA), serum protein electrophoresis, flow cytometry, and histological examinations of relevant tissues were performed as described previously. Serum creatinine was measured by ELISA using a creatinine assay kit (#ab65340, Abcam, Cambridge, MA) according to the manufacturer's protocol.

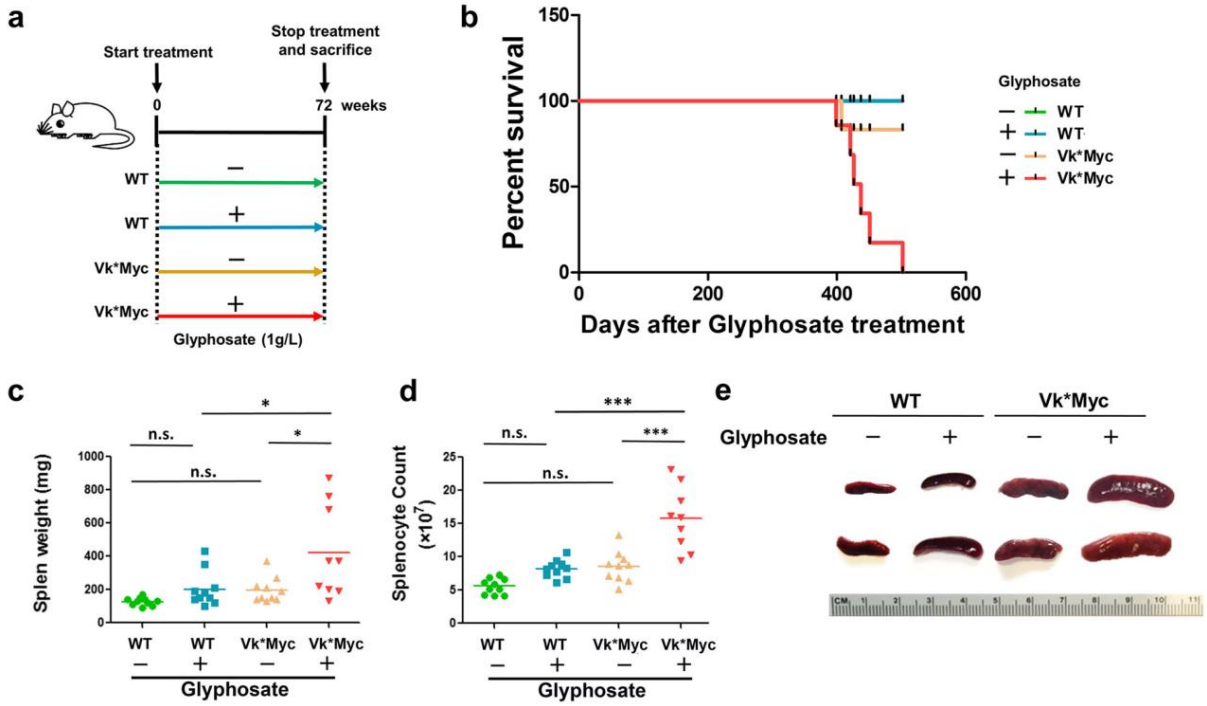
Western blotting analyses; Mouse tissues were processed for Western blotting. The antibodies were from Cell Signaling Technology (Danvers, MA, USA): AID (L7E7) (#4975) and β-actin (#3700). Blotting was run with 3 technical replicates. Horseradish peroxidase- conjugated anti-rabbit or anti-mouse IgG was used as the secondary antibody.

Statistics; Statistical analysis was carried out using GraphPad InStat 3 software (GraphPad Software, Inc., San Diego, CA, USA). The statistical significance between the groups was determined by one-way or two-way analysis of variance (ANOVA) with the appropriate post hoc testing using Tukey's test. Statistical significance was accepted at $P \leq 0.05$. All data are shown as mean ± SEM unless otherwise indicated.

Results

*Chronic glyphosate exposure reduces survival and induces splenomegaly in Vk*MYC mice*; Eight-week-old Vk*MYC mice and their WT littermates were provided 1.0 g/L glyphosate in drinking water for 72 weeks, and animals were monitored at regular intervals before sacrifice (Figure B.6.5.19.5-1a). Glyphosate significantly affected the health of Vk*MYC mice, all of which had to be euthanized by week 72 (Figure B.6.5.19.5-1b). Surviving mice in other groups were sacrificed at week 72 (at age 80 weeks) for necropsy. Inspection of organs revealed a marked increase in spleen weight and size in Vk*MYC mice treated with glyphosate compared to the other 3 groups (Figure B.6.5.19.5-1c, e). Glyphosate significantly augmented the splenocyte number in Vk*MYC mice (Figure B.6.5.19.5-1d). Findings indicate that glyphosate induces splenomegaly in both WT and Vk*MYC mice.

Figure B.6.5.19.5-1: Glyphosate reduced survival and induced splenomegaly in Vk*MYC mice. a Schematic diagram of the chronic glyphosate exposure regimen in 4 groups of mice. b The percentage of mice surviving under glyphosate exposure. The line (blue) to indicate untreated WT mice aligned directly with that for WT treated mice and so was not visible. c Mouse spleen weight at sacrifice. d The total number of splenocytes per spleen from mice at sacrifice. e Representative images of spleens from 4 groups (2 per group).



*Hematological abnormalities occur in Vk*MYC mice with chronic glyphosate exposure*; As illustrated in Fig. 2a, untreated Vk*MYC mice exhibited higher IgG levels than untreated WT mice. Upon glyphosate exposure, WT mice showed moderate yet steady increasing in IgG levels, suggesting that glyphosate induces benign monoclonal gammopathy, a mouse equivalent to human MGUS. Vk*MYC mice receiving glyphosate had greater IgG elevation, and by week 30, IgG levels jumped to 11.78 g/L, more than 5-fold the 2.07 g/L observed in untreated Vk*MYC mice. From week 36 to week 72, the mean IgG level was significantly higher in treated WT and Vk*MYC mice compared to the untreated

control groups, and Vk*MYC mice, treated or untreated, had higher IgG levels than their WT counterparts. Overt MM diagnosis was determined by serum protein electrophoresis (SPEP) analysis to detect the M-spike, which is a significant IgG monoclonal peak. SPEP results showed that Vk*MYC mice treated with glyphosate had a clear M-spike, whereas weaker M-spike was observed in glyphosate-treated WT mice. No clear M-spike was present in the untreated WT mice or Vk*MYC mice (Fig. 2b). This is the direct *in vivo* evidence that glyphosate exposure leads to M-spike, a cardinal hematological abnormality consistent with MM. Hematological abnormalities were present in glyphosate treated mice as compared to untreated control mice (Fig. 2c–i). Data support the notion that glyphosate induces multiple hematological abnormalities characteristic of MM in mice.

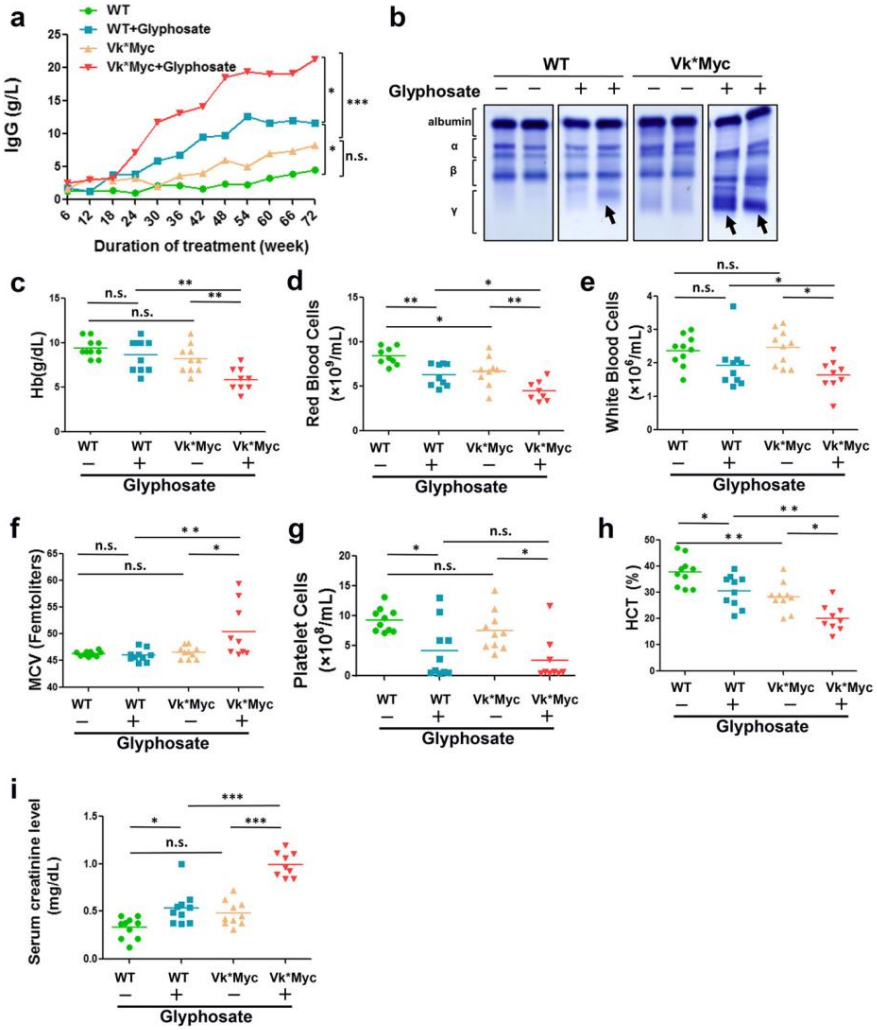


Fig. 2. Hematological abnormalities found in Vk*MYC mice treated with glyphosate. **a** Total serum IgG in mice during 72 weeks of glyphosate treatment. Mouse blood samples were collected and assayed for IgG every 6 weeks. **b** Immunoglobins from mice as determined by SPEP at week 72. Arrows indicate IgG clonal peaks (M-spike; γ-globulin peak). SPEP was performed for all mice in each group, and representative results of 2 mice per group are shown. **c**–**h** Complete blood cell counts in mice. Hemoglobin concentration (Hb, **c**), red blood cell count (**d**), white blood cell count (**e**), mean red cell volume (MCV, **f**), platelet cell count (**g**), and hematocrit (HCT, **h**) are shown. **i** Total serum creatinine in mice at week 72. The horizontal lines indicated the mean value. Data were analyzed by two-way ANOVA (**b**) or one-way ANOVA (**a**, **d**, **e**). $n = 10$ mice per group

*Vk*MYC mice chronically exposed to glyphosate develop progressive plasma cell neoplasms;* Plasma cells exhibit CD138^{hi} B220–(high CD138 expression without B220 expression). Flow cytometric analyses of cells harvested from the spleens and bone marrow showed expansion of plasma cells in mice under glyphosate exposure. A marked increase in the numbers of CD138^{hi} B220– cells was detected in both WT and Vk*MYC mice treated with glyphosate (Fig. 3a). These data demonstrate that glyphosate treatment expands the plasma cell population in the spleen and bone marrow in both WT and Vk*MYC mice.

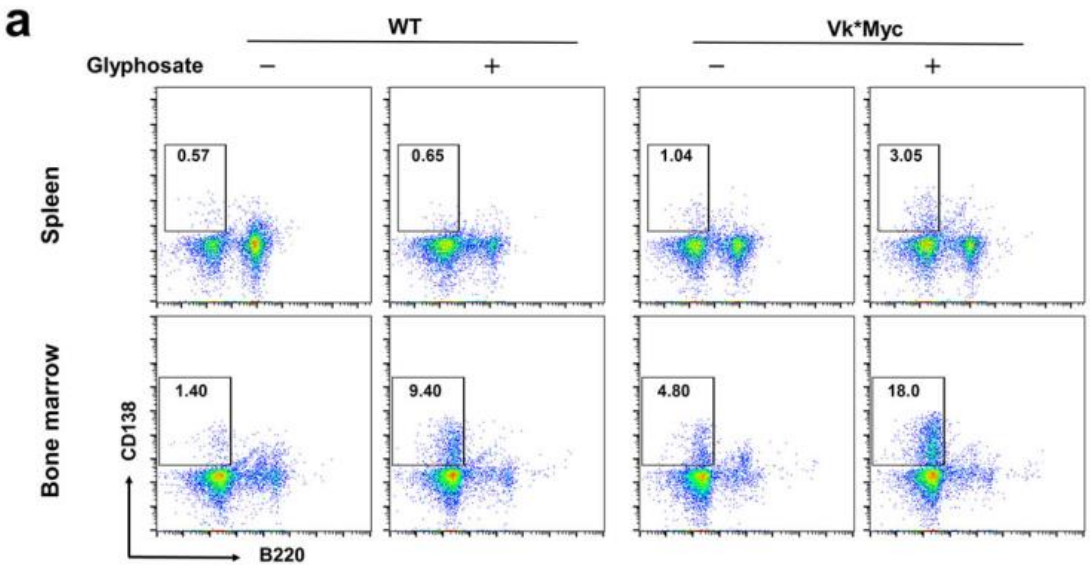


Fig. 3 Glyphosate-treated Vk*MYC mice developed progressive plasma cell neoplasms. **a** Representative flow cytometry plots detecting cell surface markers CD138 (*Y*-axis) and B220 (*X*-axis) in splenocytes (upper panel) and bone marrow cells (lower panel). The numbers on the axes denoted the $\log_{10}$ values of fluorescence. The numbers in the inserts show the percentage of $CD138^{high}B220^{-}$ cells in the entire cell population.

Chronic glyphosate exposure triggers multiple organ dysfunction; To determine whether target organ damage occurred in glyphosate-treated mice, the femoral shaft, spleen, liver, lung, and kidney were sectioned and stained with hematoxylin and eosin (H&E). Severe destructive osteolytic bone lesions in the femoral shaft were readily detectable in glyphosate-treated Vk*MYC mice. Treated WT mice showed smaller bone lesions. No lesions were observed in the control groups (Fig. 4a). Plasma cells with a perinuclear clear zone and eccentric round nucleus were observed in glyphosate-treated WT and Vk*MYC mice (Fig. 4b, c). Next, the histopathologic changes in the liver, lung, and kidney were analyzed. In glyphosate-treated mice, hepatic fibrosis and collagen deposition were observed in Vk*MYC mice, whereas WT mice showed less severe liver damage; the 2 control groups had normal hepatic tissue architectures (Fig. 4d). The lungs in treated Vk*MYC mice were severely damaged, with large distal air spaces filled by lymphocytes, neutrophils, cell debris, and hyperplastic pneumocytes; those from untreated WT mice had normal alveolar spaces and alveolar septa lined with normal pneumocytes. The lungs from treated WT mice and untreated Vk*MYC mice showed an intermediate phenotype (Fig. 4e). Renal tubular obstruction by large casts, indicative of necrotic tubular cells, were detected in glyphosate-treated WT and Vk*MYC mice, but not in the untreated groups; there were more and larger casts in treated Vk*MYC kidneys than in WT kidneys (Fig. 4f). Taken together, these data indicate that glyphosate treatment damages multiple organs in both WT and Vk*MYC mice with more severe damage occurring in Vk*MYC mice.

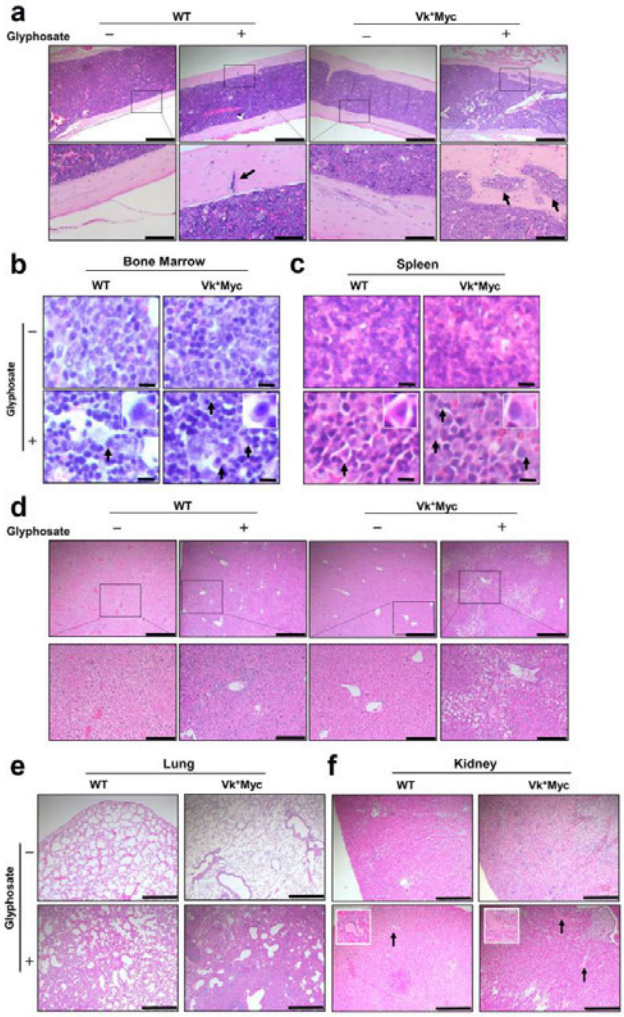


Fig. 4 Glyphosate led to multiple organ dysfunction. **a** Histological evaluation of bone morphology from 4 groups of mice. Bone lytic lesions (indicated by arrows) were detected in the femoral shaft of Vk*MYC mice treated with glyphosate. Scale bar = 500 μm (top) or 100 μm (bottom). **b** Infiltrating plasma cells in the bone marrow of glyphosate-treated mice. Scale bar = 20 μm. Arrows pointed to plasma cells. **c** Infiltrating plasma cells in the spleen of glyphosate-treated mice. Scale bar = 20 μm. Arrows point to plasma cells. **d** Collagen deposition in the liver was observed in glyphosate-treated Vk*MYC mice. *n* = 10 mice per group. Scale bar = 500 μm (top) or 200 μm (bottom). **e** Destruction of lung morphology was observed in glyphosate-treated Vk*MYC mice. *n* = 10 mice per group. Scale bar = 500 μm. **f** Protein deposition (indicated by arrows) in the kidney was observed in glyphosate-treated Vk*MYC mice. *n* = 10 mice per group. Scale bar = 500 μm. All panels show 1 representative image each from 4 groups of mice unless otherwise indicated

Chronic glyphosate exposure induces AID upregulation; To investigate the underlying mechanisms of glyphosate-mediated MGUS induction and MM progression, expression of activation-induced cytidine deaminase (AICDA, also known as AID) in mice treated with 1.0 g/L glyphosate for 72 weeks was examined. It was found that AID was upregulated in both the bone marrow and the spleen of WT and Vk*MYC mice (Fig. 5a).

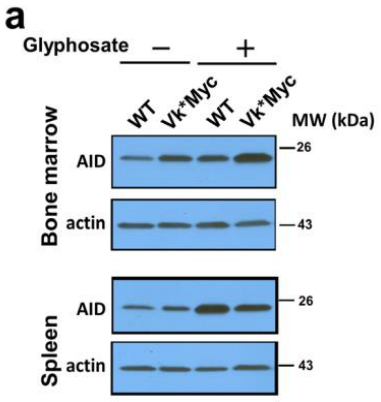


Fig. 5 Glyphosate-induced AID upregulation. **a** Western blotting analysis of mice treated with 1.0 g/L of glyphosate for 72 weeks.

Acute glyphosate exposure induces AID upregulation; To determine the acute effect of glyphosate, 8-week-old WT and Vk*MYC mice were treated with increasing doses of glyphosate (1, 5, 10, and 30 g/L) in drinking water for 7 days. This acute treatment neither increased spleen weight nor affected body weight significantly. Next, expression of AID in the spleen, bone marrow, and lymph nodes were analysed. It was found that AID was upregulated in a glyphosate dose-dependent manner in the spleen and bone marrow of WT and Vk*MYC mice treated with 10 and 30 g/L of glyphosate (Fig. 5c). Given the role of AID in MM pathogenesis in the context of its capacity to induce mutations and chromosome translocations, these results from mice with chronic and acute glyphosate treatment support an AID mediated mutational mechanism in the etiology of MGUS and MM under glyphosate exposure.

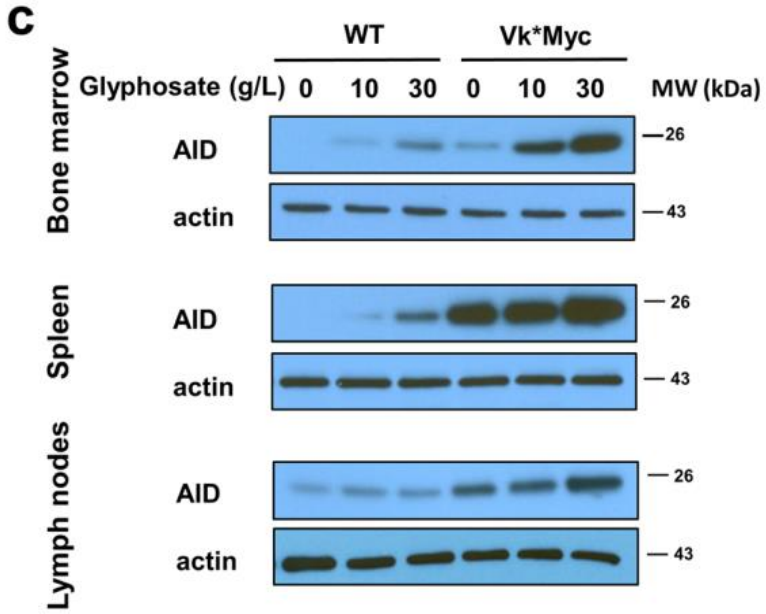


Fig. 5c Western blotting analysis of mice treated with glyphosate for 7 days. One representative mouse

Discussion

In this study, it was demonstrated that glyphosate induces benign monoclonal gammopathy (mouse equivalent to MGUS in human) in WT mice and promotes MM progression in Vk*MYC mice. In Vk*MYC mice, glyphosate causes hematological abnormalities like anemia and multiple organ dysfunction like lytic bone lesions and renal damage, which are hallmarks of human MM. Beyond epidemiology and animal models, the mechanism of action is the third pillar required to define a compound as a carcinogen. Numerous studies have revealed that glyphosate may induce DNA damage, oxidative stress, inflammation, and immunosuppression, as well as modulate cell proliferation and death and disrupt sex hormone pathways. However, these mechanistic studies have failed to explain why glyphosate exposure is only positively associated with MM and NHL. Our results demonstrate that glyphosate treatment, either at a chronic low dose or acute high doses, upregulates the expression of AID in the bone marrow and spleen of both WT and Vk*MYC mice. The data disclose, for the first time, that glyphosate elicits a B cell-specific mutational mechanism of action in promoting carcinogenesis, as well as offering experimental evidence to support the epidemiologic finding regarding its tissue specificity in carcinogenesis (i.e. only increasing the risk for MM and NHL). The "acceptable daily intake (ADI)" of glyphosate currently allowed in the USA, defined as the chronic reference dose as determined by EPA, is 1.75 mg/kg body weight/day; an average adult male or female in the USA who weighs 88.8 or 76.4 kg and drinks 2 L (8 glasses) water daily containing 77.7 (for male) or 66.9 (for female) mg/L glyphosate would reach the ADI. A dose of 1,000 mg/L glyphosate in drinking water (~ 15-fold the ADI) was chosen in this study, which caused significant adverse effects and accelerated

MM progression in Vk*MYC mice, i.e. animals predisposed to MM.

Conclusion
The data provide *in vivo* evidence to support that glyphosate induces MGUS and promotes disease progression to MM. A B cell-specific mutational mechanism for glyphosate exposure that increases MM and NHL risk was uncovered, providing a molecular basis for human epidemiological findings.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The objective of this study was to investigate the pathogenic role of glyphosate in multiple myeloma using Vk*MYC mice. The study did demonstrate the ability of glyphosate to impact measured parameters in the tested models. However, this study is not appropriate for human health risk assessment. The number or animals per group was below the recommended number for guideline toxicity studies and to perform sufficient statistical analysis. Only one dose level was used in the chronic study. It was not possible to correlate effects with a glyphosate dose-response as the water consumption (and therefore test substance intake) of animals was not provided and it is therefore impossible to calculate a dose on a mg/kg bw basis for risk assessment.

This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, only one dose was considered for the chronic study and the number of animals used per group was either too low (acute study) or not reported (chronic study).

Reliability criteria for *in vivo* toxicology studies

Publication: Wang *et al*., 2019	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y?	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Purity not reported. Source: Sigma-Aldrich, St Louis, USA.
Only glyphosate acid or one of its salts is the tested substance		
AMPA is the tested substance		
Study		
Test species clearly and completely described	Y	Wild-type (WT) C57Bl/6 mice and Vk*MYC mice.
Test conditions clearly and completely described		
Route and mode of administration described	Y	Oral *via* drinking water.
Dose levels reported	Y	For the chronic study: 1 g/L in drinking water for 72 weeks. For the acute study: 1, 5, 10, 30 g/L for 7 days.
Number of animals used per dose level reported	Y	5/group for the acute study, no/group not reported for the chronic study.
Method of analysis described for analysis test media	N	
Validation of the analytical method	N	
Analytical verifications of test media	N	
Complete reporting of effects observed	Y	

Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	N	Not possible. Only one dose level used.
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for the risk assessment of glyphosate but reliable with restrictions because the glyphosate used was not characterized, only one dose was considered for the chronic study and the number of animals used per group was either too low (acute study) or not reported (chronic study).		

Assessment and conclusion by RMS:

While the RMS agrees with the applicants that no exact intake values can be calculated due to the lack of water consumption data default conversion factors can be applied to estimate the daily intake. The EFSA Guidance on default values to be used in the absence of measured data (EFSA Journal 2012 ; 10(3) : 2579) provides conversion factor to calculate an estimated daily dose from concentrations of substances in drinking water. A conversion factor of 0.09 is recommended in this Guidance for chronic studies and 0.18 for the subacute studies with mice.
- Then the daily intake for the chronic study is 90 mg/kg bw/day (1 g glyphosate/L x 0.09 = 0.09 g/kg bw/day).
- An then the daily intake for the subchronic study is 180 mg/kg bw/day, 900 mg/kg bw/day, 1800 mg/kg bw/day and 5400 mg/kg bw/day (1, 5, 10 and 30 g/L multiplied by 0.18).
It is noted that for the chronic study the selected dose level is low compared to the guideline toxicity studies conducted in mice, which makes it surprising that effects on haematological parameters and organ toxicity was observed in WT C57BL/6 mice at this dose level while the NOAELs in the guideline chronic studies are far higher.

We agree with the assessment made by the applicants that the number of animals used in the study are quite low (n=5 for the subacute study). The number of animals for the chronic study is not clearly reported in the study. Based on the individual data points in the result figures it seems that 10 animals were included per group which is low compared to OECD requirements for a chronic toxicity study (20/sex/dose, OECD 452).

Based on the low number of animals it is agreed that the study is reliable with restrictions.

Considering the low number of animals and the remarkably low dose levels in which effects are observed there uncertainties regarding the liability of the study and therefore the study is not considered to directly impact the overall assessment of glyphosate.

It is noted that no association with MGUS was observed in humans in the Agricultural Health Survey (see Landgren, 2009 reported in B.6.5.18.27)

B.6.5.18.10. Supporting publications – Andreotti, 2018

Data point:	CA 5.5/035
Report author	Andreotti, G. *et al.*
Report year	2018
Report title	Glyphosate Use and Cancer Incidence in the Agricultural Health Study
Document No	doi: 10.1093/jnci/djx233 ISSN: 0027-8874
Guidelines followed in study	Not applicable

Deviations from current test guideline	Not applicable
Previous evaluation :	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable/Reliable with restrictions Conclusion AGG: Study reliable.

Full summary of the study according to OECD format

In 2005, an evaluation of glyphosate and cancer risk was conducted in the Agricultural Health Study (AHS) (DeRoos *et al*. 2005). This evaluation considered glyphosate use reported at enrollment (1993–1997) and included 2,088 cancers diagnosed between enrollment and 2001. No statistically significant associations were found for any cancer site. For NHL, the relative risk (RR) adjusted for age, personal factors and other pesticides was 1.1 (95 % CI 0.7-1.9) and there was no trend of increasing RRs with increasing frequency of glyphosate. For multiple myeloma, the overall adjusted RR was 2.6 (95 % CI 0.7 – 9.4) and the assessment of RR by frequency of glyphosate use did not evidence a significant trend. Andreotti *et al*. 2018 updated the 2005 AHS publication by DeRoos *et al*. (2005), extending cancer incidence follow-up through 2012 in North Carolina and 2013 in Iowa and incorporating additional exposure information from a follow-up questionnaire. The authors also dealt with missing information through imputation and conducted sensitivity analyses to address the potential for various types of bias in their primary analyses. This 2018 publication includes a total of 7,290 cancers, 3.6 times as many as in the earlier publication. The median lifetime days of glyphosate use for cohort members who reported glyphosate use (83 % of the cohort) was 48 days (interquartile range (IQR) 20 to 166 days). The authors found no evidence of an association between glyphosate use and risk of any solid tumour, NHL (RR 0.87 (95 % CI 0.64-1.20 in the highest intensity weighted exposure quartile, p_{trend} 0.95), or multiple myeloma (RR 0.87, 95 % CI 0.45-1.69 highest quartile, p_{trend} 0.84). They found a moderately elevated RR for acute myelogenous leukaemia for the highest exposure quartile that was not statistically significant ($RR_{quartile\ 4}$ = 2.44, 95 % CI 0.94-6.32, p_{trend} = 0.11) but was statistically significant when a 20-year lag period was considered ($RR_{tertile\ 3}$ = 2.04, 95%-CI = 1.05-3.97, p_{trend} = 0.04). The findings for cancer types were consistent across different exposure metrics, in various sensitivity analyses, and for lagged exposure analyses meant to address cancer induction-latency.

Materials and methods

Study design

Briefly, 57,310 individuals seeking licenses to apply restricted-use pesticides were enrolled between 1993 and 1997. Of the enrolled participants, 63% completed a follow-up phone interview approximately five years after enrollment (1999–2005). Incident cancer diagnoses were ascertained *via* linkage to cancer registries in Iowa (through 2013) and North Carolina (through 2012). Cancer diagnoses were classified according the International Classification of Disease– Oncology, 3rd Revision. Subtypes of lymphoid malignancies were defined according to the Surveillance, Epidemiology, and End Results Program Lymphoma Subtype Recodes. According to this updated classification of lymphoid malignancies, multiple myeloma was included in the analyses as a subtype of non-Hodgkin's lymphoma. Vital status was ascertained *via* state mortality registries and the National Death Index, and state of residence was regularly updated using various government databases.

Exposure assessment

Lifetime use of glyphosate and 49 other pesticides was ascertained at enrollment and in the follow-up questionnaire. At enrollment, applicators reported the number of years and days per year each pesticide was used, while at follow-up applicators reported the number of days each pesticide was used in the most recent year farmed. Using this information, three metrics of cumulative lifetime exposure were created for each pesticide: ever/never use, lifetime days of use (days per year multiplied by the number of years), and intensity-weighted lifetime days (lifetime days multiplied by an intensity score). The intensity score was derived from an algorithm based on literature-based measurements and information provided by the applicator, specifically whether the participant mixed or applied pesticides, repaired pesticide-related equipment, used personal protective equipment, and application method used. For participants who did not complete the follow-up questionnaire (37 %), multiple imputation was used to impute pesticide use since enrollment. Factors used to impute pesticide use included demographic data and medical history, as well as factors related to farm characteristics and reported pesticide use

at enrollment.

Statistical analysis

For this analysis, individuals who had a history of cancer at enrollment (n=1096), did not live in North Carolina or Iowa (n=343), or did not report whether they had used glyphosate or not at enrollment (n=1620) were excluded, resulting in an analytic sample of 54,251 licensed farmers and applicators. Individuals accumulated person-time from enrollment until the earliest of the following events: movement out of state, diagnosis of cancer, death, or end of the follow-up period (December 31, 2012 in NC, December 31, 2013 in IA). Poisson regression was used to calculate incidence rate ratios (RRs) and 95 % confidence intervals (CIs), and PROC MIANALYZE was used to obtain the appropriate variance for the imputed data. All statistical significance tests were two-sided and considered to be statistically significant when $p \leq 0.05$. RRs for total cancer and for cancer sites with at least 20 exposed cases were evaluated. For analyses by exposure level, based on the distribution among all cancer cases, cumulative lifetime days and intensity-weighted lifetime days of glyphosate exposure into quartiles, tertiles, or the median were categorized, such that there were at least five exposed cases in each category. Linear trend was evaluated according to the Wald test using the median of each exposure category as a continuous variable. Risk estimates were adjusted for attained age (continuous), cigarette smoking status (never, former, current), alcohol drinks per month (none, ≤ 6 per month, ≥ 7 per month), family history of any cancer (yes, no), state of recruitment (North Carolina, Iowa), and the five pesticides most highly correlated with glyphosate based on lifetime days and intensity-weighted lifetime days (r > 0.4: atrazine, alachlor, metolachlor, trifluralin, 2,4-D). Lagged exposure was also evaluated allowing for 5, 10, 15, or 20 years to address the induction-latency period for specific cancers. Other potential confounding factors were calculated, including body mass index (BMI; <25, 25–<30, _30 kg/m^2) and pack-years of cigarettes smoked (tertiles of use among former and current smokers). The numbers of women and nonwhites were small, precluding adjustment for sex and race for most cancer sites; in sensitivity analyses, the risks in men and whites alone were assessed. For lymphohematopoietic cancers, RRs were additionally adjusted for occupational exposure to solvents, gasoline, x-ray radiation, and engine exhaust, and pesticides linked to lymphohematopoietic malignancies in previous AHS analyses (lindane, DDT, diazinon, terbufos, and permethrin). The risk of NHL excluding multiple myeloma was calculated for comparison with previously published studies. Lastly, sensitivity analyses were conducted to evaluate the impact of including additional exposure information.

Results

Among 54,251 participants, 44,932 (82.8 %) reported ever using glyphosate at enrollment or during follow-up. Among the participants who used glyphosate, the median lifetime days of use was 48 (interquartile range [IQR] = 20–166 days), and the median lifetime years of use was 8.5 years (IQR = 5–14 years). A total of 7,290 incident cancers were diagnosed during the follow-up period. Among the participants who used glyphosate and were diagnosed with cancer during follow-up (n=5,779), the median lifetime days of use was 38.75 (IQR = 13.75–108.5 days), and the median lifetime years of use was 8.0 (IQR = 3.5–13.0). Selected characteristics of the study participants by glyphosate use are presented in Table 1 (below). Risk ratios for intensity-weighted lifetime days of glyphosate use and cancer risk are shown in Table 2. Glyphosate use was not associated with total cancer or with lymphohematopoietic malignancies. There also was no evidence for positive associations with NHL (RR in the highest intensity weighted days of glyphosate use quartile = 0.9 (95 % CI 0.6 – 1.2)), multiple myeloma ($RR_{quartile\ 4}$ = 0.9, 95 % CI 0.5 – 1.7) or for any NHL subtype. Although not statistically significant, the authors observed an elevated RR for acute myeloid leukemia (AML; n = 57 exposed cases) among applicators in the highest quartile of intensity weighted glyphosate use compared with never users (n = 18 cases, RR.2.4, 95 % CI = 0.9 to 6.3, P_{trend} =.11). The results based on intensity weighted days of use were very similar to results based on unweighted days of use.

The impact of lagging exposure on risk estimates for lymphohematopoietic cancers was evaluated for intervals of 5, 10, 15 and 20 years. The patterns of risk for lagged exposures were similar to those for unlagged exposures. For AML the rate ratio was elevated and the trend statistically significant with a 20 year lag and tertiles of exposure (RR 2.04, 95% CI 1.05-3.97).

Conclusion

In conclusion, the authors found no evidence of an association between glyphosate use and risk of any solid tumor, NHL, or multiple myeloma. They found an elevated RR for AML that merits evaluation in AHS updates or other studies considering the observed consistent pattern of increasing risk with increasing exposure and the statistically significant trend with lagged exposure of 10 or more years. This findings across cancer types were consistent across different exposure metrics, in various sensitivity analyses, and for lagged exposure analyses meant to address cancer induction-latency.

Table 1. Selected characteristics of the Agricultural Health Study population by glyphosate use

Characteristics*	Never-used glyphosate No. (%)	Lifetime days of glyphosate use† < Median No. (%)	Lifetime days of glyphosate use† ≥ Median No. (%)
Total	9319 (100.0)	19 714 (100.0)	24 727 (100.0)
Age at enrollment, y			
<30	814 (8.7)	1726 (8.8)	2372 (9.6)
30–39	1730 (18.6)	4293 (21.8)	6612 (26.7)
40–49	2217 (23.8)	5304 (26.9)	7437 (30.1)
50–59	2051 (22.0)	4261 (21.6)	4759 (19.2)
60–69	1797 (19.3)	3043 (15.4)	2738 (11.1)
70+	710 (7.6)	1087 (5.5)	809 (3.3)
Sex			
Male	8887 (95.4)	19 220 (97.5)	24 203 (97.9)
Female	432 (4.6)	494 (2.5)	524 (2.1)
Race			
White	8838 (94.8)	19 128 (97.0)	24 267 (98.1)
Black and other	441 (4.7)	538 (2.7)	404 (1.6)
Missing	40 (0.4)	48 (0.2)	56 (0.2)
State of recruitment			
Iowa	6692 (71.8)	12 668 (64.3)	15 756 (63.7)
North Carolina	2627 (28.2)	7046 (35.7)	8971 (36.3)
Applicator type			
Private (farmer)	8476 (91.0)	18 717 (94.9)	21 932 (88.7)
Commercial	843 (9.0)	997 (5.1)	2795 (11.3)
Highest level of education			
High school or less	6528 (70.1)	11 409 (57.9)	12 005 (48.6)
Beyond high school	2569 (27.6)	7884 (40.0)	12 213 (49.2)
Missing	222 (2.4)	421 (2.1)	509 (2.1)
Body mass index, kg/m^2			
<25	1656 (17.8)	3779 (19.2)	4168 (16.9)
25–<30	3044 (32.7)	7123 (36.1)	8492 (34.3)
30+	1435 (15.4)	3175 (16.1)	3985 (16.1)
Missing	3184 (34.2)	5637 (28.6)	8082 (32.7)
Cigarette smoking status			
Never	4987 (53.5)	10 371 (52.6)	12 876 (52.1)
Former	2621 (28.1)	6004 (30.5)	7295 (29.5)
Current	1526 (16.4)	3147 (16.0)	4355 (17.6)
Missing	185 (2.0)	192 (1.0)	201 (0.8)
Cigarette smoking pack-years			
Never	4987 (53.5)	10 371 (52.6)	12 876 (52.1)
Former, tertile 1	896 (9.6)	2004 (10.2)	2471 (10.0)
Former, tertile 2	791 (8.5)	1865 (9.5)	2198 (8.9)
Former, tertile 3	741 (8.0)	1748 (8.9)	2109 (8.5)
Current, tertile 1	548 (5.9)	1037 (5.3)	1513 (6.1)
Current, tertile 2	453 (4.9)	975 (4.9)	1399 (5.7)
Current, tertile 3	461 (4.9)	1076 (5.5)	1376 (5.6)
Missing	442 (4.7)	638 (3.2)	785 (3.2)
Usual number of alcohol drinks per month in year prior enrollment			
Never	3150 (33.8)	6406 (32.5)	6946 (28.1)
≤6/mo	3036 (32.6)	6646 (33.7)	8240 (33.3)
≥7/mo	2492 (26.7)	5631 (28.6)	8646 (35.0)
Missing	641 (6.9)	1030 (5.2)	895 (3.6)
Family history of cancer			
No	5452 (58.5)	10 846 (55.0)	13 866 (56.1)
Yes	3226 (34.6)	7700 (39.1)	9876 (39.9)
Missing	641 (6.9)	1168 (5.9)	985 (4.0)

*Data from the enrollment questionnaire.
†Based on median cumulative lifetime days of glyphosate use among all cancer cases (38.75 days)

Table 2. Cancer incidence in relation to intensity-weighted lifetime days of glyphosate use in the Agricultural Health Study

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
All cancers				
	None	1511	1.00 (reference)	
	Q1	1445	0.99 (0.91 to 1.07)	
	Q2	1443	0.99 (0.91 to 1.07)	
	Q3	1440	1.04 (0.96 to 1.13)	
	Q4	1451	0.99 (0.91 to 1.08)	.91
Oral cavity				
	None	33	1.00 (reference)	
	Q1	36	0.95 (0.56 to 1.60)	
	Q2	35	0.92 (0.54 to 1.57)	
	Q3	35	0.96 (0.56 to 1.65)	
	Q4	35	0.84 (0.48 to 1.46)	.54
Colon				
	None	116	1.00 (reference)	
	Q1	104	1.00 (0.74 to 1.35)	
	Q2	102	1.03 (0.76 to 1.39)	
	Q3	102	1.06 (0.78 to 1.44)	
	Q4	96	1.01 (0.74 to 1.38)	1.00
Rectum				
	None	50	1.00 (reference)	
	Q1	43	0.81 (0.51 to 1.28)	
	Q2	55	1.16 (0.76 to 1.76)	
	Q3	39	0.80 (0.50 to 1.29)	
	Q4	46	0.84 (0.52 to 1.34)	.43
Pancreas				
	None	25	1.00 (reference)	
	Q1	42	1.80 (1.05 to 3.08)	
	Q2	42	1.69 (0.98 to 2.94)	
	Q3	24	1.09 (0.59 to 2.02)	
	Q4	23	1.06 (0.57 to 1.97)	.14
Lung				
	None	144	1.00 (reference)	
	Q1	117	0.92 (0.70 to 1.22)	
	Q2	138	1.19 (0.91 to 1.56)	
	Q3	159	1.39 (1.07 to 1.82)	
	Q4	131	1.00 (0.76 to 1.33)	.78
Melanoma				
	None	56	1.00 (reference)	
	Q1	59	1.00 (0.67 to 1.50)	
	Q2	67	1.18 (0.80 to 1.74)	
	Q3	69	1.12 (0.75 to 1.67)	
	Q4	78	1.17 (0.78 to 1.74)	.53
Prostate				
	None	579	1.00 (reference)	
	Q1	571	0.99 (0.87 to 1.12)	
	Q2	564	0.95 (0.83 to 1.08)	
	Q3	559	1.03 (0.91 to 1.18)	
	Q4	571	0.99 (0.86 to 1.13)	.89
Testicular				
	None	7	1.00 (reference)	
	T1	17	1.28 (0.49 to 3.34)	
	T2	12	0.74 (0.26 to 2.09)	
	T3	11	0.57 (0.20 to 1.67)	.07
Bladder				
	None	66	1.00 (reference)	
	Q1	86	1.29 (0.91 to 1.82)	
	Q2	68	1.04 (0.72 to 1.51)	
	Q3	66	1.09 (0.75 to 1.59)	
	Q4	79	1.26 (0.87 to 1.82)	.42
Kidney				
	None	54	1.00 (reference)	
	Q1	54	1.13 (0.74 to 1.71)	
	Q2	50	0.91 (0.59 to 1.41)	
	Q3	45	0.87 (0.55 to 1.38)	
	Q4	53	1.03 (0.66 to 1.61)	.95
Lymphohematopoietic				
	None	161	1.00 (reference)	
	Q1	136	0.87 (0.64 to 1.19)	
	Q2	126	0.88 (0.66 to 1.17)	
	Q3	137	0.93 (0.71 to 1.23)	
	Q4	144	1.00 (0.74 to 1.34)	.43
Hodgkin lymphoma				
	None	7	1.00 (reference)	
	M1	7	0.59 (0.17 to 2.11)	
	M2	11	0.90 (0.25 to 3.24)	.94
Non-Hodgkin lymphoma				
	None	135	1.00 (reference)	
	Q1	113	0.83 (0.59 to 1.18)	
	Q2	104	0.83 (0.61 to 1.12)	
	Q3	112	0.88 (0.65 to 1.19)	
	Q4	111	0.87 (0.64 to 1.20)	.95
Non-Hodgkin lymphoma B cell				
	None	128	1.00 (reference)	
	Q1	102	0.79 (0.55 to 1.13)	
	Q2	93	0.76 (0.56 to 1.05)	
	Q3	106	0.88 (0.64 to 1.21)	
	Q4	103	0.86 (0.62 to 1.19)	.86
Chronic lymphocytic lymphoma, small lymphocytic leukemia				
	None	36	1.00 (reference)	
	Q1	28	0.75 (0.40 to 1.41)	
	Q2	26	0.76 (0.41 to 1.41)	
	Q3	26	0.90 (0.50 to 1.62)	
	Q4	27	0.87 (0.48 to 1.58)	.71
Diffuse large B cell lymphoma				
	None	27	1.00 (reference)	
	Q1	28	1.11 (0.60 to 2.07)	
	Q2	23	0.94 (0.49 to 1.80)	
	Q3	30	1.13 (0.59 to 2.17)	
	Q4	22	0.97 (0.51 to 1.85)	.83
Marginal-zone lymphoma				
	None	4	1.00 (reference)	
	M1	6	0.39 (0.06 to 2.45)	
	M2	5	0.44 (0.09 to 2.17)	.67
Follicular lymphoma				
	None	16	1.00 (reference)	
	T1	21	0.89 (0.37 to 2.15)	
	T2	11	0.61 (0.23 to 1.60)	
	T3	20	0.85 (0.36 to 2.03)	.95
Multiple myeloma				
	None	30	1.00 (reference)	
	Q1	19	0.70 (0.36 to 1.36)	
	Q2	26	0.94 (0.50 to 1.76)	
	Q3	19	0.78 (0.39 to 1.56)	
	Q4	24	0.87 (0.45 to 1.69)	.84
Non-Hodgkin lymphoma T cell				
	None	2	1.00 (reference)	
	M1	14	4.25 (0.73 to 24.64)	
	M2	6	1.53 (0.23 to 10.38)	.31

Table 2. (continued)

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
Acute myeloid leukemia				
	None	9	1.00 (reference)	
	Q1	13	1.62 (0.60 to 4.38)	
	Q2	14	1.70 (0.61 to 4.73)	
	Q3	12	1.46 (0.49 to 4.37)	
	Q4	18	2.44 (0.94 to 6.32)	.11
Chronic myeloid leukemia				
	None	7	1.00 (reference)	
	M1	5	0.36 (0.09 to 1.43)	
	M2	11	0.82 (0.23 to 2.98)	.36

*Cancer sites are based and presented in order of Surveillance, Epidemiology, and End Results Site Recode ICD-O-3. CI = confidence interval; RR = rate ratio.
†Quartiles: Q1: 1–598.9; Q2: 599–1649.9; Q3: 1650–4339.9; Q4: ≥4340.0. Tertiles: T1: 1–866.24; T2: 866.25–2963.9; T3: ≥2964.0. Median: M1: 1–1649.9; M2: ≥1650.0.
‡Poisson regression was used to model rate ratios and confidence intervals, and *P* values were calculated using a two-sided Wald test. All models adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, 2,4-D.

Table 3. Cancer incidence in relation to lagged intensity weighted lifetime days of glyphosate use in the Agricultural Health Study

		5-y lag			20-y lag		
Cancer sites*	Glyphosate use†	No. of cases	RR (95% CI)‡	P_{trend}‡	No. of cases	RR (95% CI)‡	P_{trend}‡
Acute myeloid leukemia							
	None	10	1.00 (reference)		34	1.00 (reference)	
	Q1/T1	12	1.35 (0.55 to 3.31)		8	1.26 (0.57 to 2.76)	
	Q2/T2	13	1.59 (0.63 to 4.01)		9	1.33 (0.62 to 2.84)	
	Q3/T3	13	1.47 (0.54 to 3.96)		15	2.04 (1.05 to 3.97)	.04
	Q4	18	2.32 (0.98 to 5.51)	.07	–	–	
Chronic myeloid leukemia							
	None	8	1.00 (reference)		16	1.00 (reference)	
	M1	4	0.31 (0.07 to 1.29)		3	0.58 (0.13 to 2.63)	
	M2	11	1.00 (0.32 to 3.18)	.29	4	0.87 (0.24 to 3.23)	.91

*Cancer sites are based and presented in order of Surveillance, Epidemiology, and End Results Site Recode ICD-O-3. CI = confidence interval; RR = rate ratio.
†Five-year lag quartiles: Q1: 1–530.9; Q2: 531.0–1511.9; Q3: 1512.0–4063.4; Q4: ≥4063.5. Five-year lag tertiles: T1: 1–787.4; T2: 787.5–2795.9; T3: ≥2796.0. Five-year lag median: M1: 1–1511.9; M2: ≥1512.0. Twenty-year lag quartiles: Q1: 1–281.3; Q2: 281.4–895.9; Q3: 896–2609.9; Q4: ≥2610.0. Twenty-year lag tertiles: T1: 1–409.4; T2: 409.5–1819.9; T3: ≥1820.0. Twenty-year lag median: M1: 1–895.9; M2: ≥896.0.
‡Poisson regression was used to model rate ratios and confidence intervals, and *P* values were calculated using a two-sided Wald test. All models were adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, 2,4-D.

Assessment and conclusion by applicant:

The AHS is an ongoing prospective cohort study of glyphosate and other pesticides. It was initiated in 1993 and has been ongoing for more than 25 years. Researchers from the US National Cancer Institute and other government agencies initiated the AHS as a prospective cohort study to eliminate the possibility of case-recall bias – an intractable potential bias in case control studies that rely on self-reported exposure information. Crump (Risk Analysis DOI:10.1111/risa.13440) has recently illustrated that the results from the glyphosate case-control studies align closely with what would be expected from case recall bias.

In addition to obviating concerns about case-recall bias, the Andreotti *et al*. publication is noteworthy on several counts. First, the frequency of glyphosate use by participants (median = 48 days, IQR 20 to 166 days) vastly exceeds that in the glyphosate case-control studies. In those studies the most frequent days of use category is > 10 days (Eriksson M, *et al*. Int J Cancer. 2008; 123:1657-1663), while most of the case control studies' primary analyses were based on 1 day or more of use in a lifetime. Second, the participants in the AHS were licensed pesticide applicators who were considered by the authors to be very capable to report pesticide use accurately compared with other study populations. Third, the analyses by Andreotti *et al*. controlled for a multitude of personal factors and for other pesticides in addition to incorporating a wide range of sensitivity and lagged analyses (allowing for up to 20+ years induction-latency). No other study has evaluated the relationship between glyphosate use and cancers as extensively. The AHS is, by far, the most informative and relevant study epidemiologic study for glyphosate to date. The authors found no evidence of an association between glyphosate use and risk of any solid tumour, NHL, or multiple myeloma.

Accordingly, given the AHS results for NHL among those with extensive glyphosate use (n = 111 exposed cases, RR = 0.9, 95 % CI 0.6 – 1.2), it is unlikely that the positive associations for glyphosate and NHL in some case control studies are valid. As follow-up of the AHS cohort continues, it remains to be seen whether subsequent results will identify relationships between individual cancers and glyphosate use that are relevant for risk evaluations.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Andreotti G. *et al.,* 2018	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	Yes	
Appropriate study population to address potential glyphosate-related health outcomes	Yes	Most appropriate population studied to date. Highest frequency of glyphosate use by far.
Exposure studied		
Exposure to formulations with glyphosate as a.s.	Yes	
Exposure to formulations with other a.s.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	No	
Adequate statistical analysis	Yes	Very comprehensive
Adequate consideration of personal confounding factors	Yes	Very comprehensive
Adequate consideration of potentially confounding exposures	Yes	Very comprehensive
Overall assessment		
Reliable without restrictions	Yes	Most reliable epidemiology study for glyphosate users versus non-users.
Reliable with restrictions	Yes	Certain analyses are limited: dose is not known, only frequency of use. So, "dose response" analyses must be interpreted cautiously.
Not reliable	No	

Assessment and conclusion by RMS:
The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability high - prospective cohort study, well described study design
Population: Reliability high – anybody seeking licenses for pesticide spraying between 1993 and 1997 was invited. No potential for selection bias.
Exposure assessment: Reliability high – pesticide use was determined at enrolment (no issues for recall bias) and in a follow-up questionnaire after 5 years. All participants were licensed applicators who are likely to report their exposure reliably.
Outcome assessment: Reliability high - cancer incidents based on cancer registries.
Confounder control : Reliability high – risk estimates were adjusted for attained age, BMI, cigarette smoking status, packs of cigarettes smoked, alcohol drinks per month, family history of cancer, state of recruitment and the five pesticides most highly correlated with glyphosate.
Statistical methods : Reliability high, appropriate statistical methods used, large study population, adjustments made for confounders, extensive analysis conducted including intensity-weighted lifetime days of glyphosate use as well as lagged exposure.
Reporting : Reliability high, key elements of the material and methods and results section are reported in sufficient details.

Overall, the reliability of the study is concluded to be high. Glyphosate use was not associated with total cancer or the vast majority of cancers investigated including NHL which has previously been reported in some case-control studies.

Exposure assessment is based on self-report instead of actual dose, therefore as the authors noted as well, dose-response relationships must be carefully interpreted. In general, cohort studies are not prone to recall bias, however, the questionnaire itself contains questions that could entail recall bias, especially those that were used for exposure measurement matrices (e.g. questions on the use of specific pesticides). This is acknowledged by the authors that nondifferential misclassification bias may occur.

The study did find an elevated RR for acute myeloid leukaemia in the highest quartile of exposure (RR = 2.44, 95%-CI 0.94-6.32, p_{trend} 0.11). The effect was not statistically significant, although the RR was significant when a 20-year lag period and tertiles of exposure was taken into account ($RR_{tertile\ 3}$ = 2.04, 95%-CI 1.05-3.97, p_{trend} = 0.04). As reported by the study authors an association between glyphosate exposure and acute myeloid leukaemia has not been previously reported in other epidemiological studies and merits further evaluation. However, it should be noted that a low number of cases was included in this subgroup (n = 15). The RMS adds that also for non-Hodgkin's lymphoma of T cell subtype (NHL) an elevated risk ratio was found for the 20-year lagged exposure (NHL: RR of 2.97, 95% CI: 1.20-7.31). However, also here it should be noted that a low number of cases was included in this subgroup (n = 9).

It is noted that a high number of cancer sites were analysed so there is the possibility of statistical findings by chance.

B.6.5.18.11. Supporting publications – Presutti, 2016

Data point:	CA 5.5/036
Report author	Presutti, R. *et al.*
Report year	2016
Report title	Pesticide exposures and the risk of multiple myeloma in men: An analysis of the North American Pooled Project
Document No	DOI: 10.1002/ijc.30218 ISSN: 0020-7136
Guidelines followed in study	None
Deviations from current test guideline	No
Previous evaluation	No, submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	Non-GLP

Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Reliable with restrictions

Full summary of the study according to OECD format

Three case-control studies in the United States and Canada were pooled to create the North American Pooled Project (NAPP) to investigate associations between pesticide use and haematological cancer risk. This analysis used data from 547 MM cases and 2700 controls. Pesticide use was evaluated as follows: ever/never use; duration of use (years); and cumulative lifetime- days (LD) (days/year handled 3 years of use). Odds ratios (ORs) and 95 % confidence intervals (CIs) were estimated using logistic regression adjusted for age, province/state of residence, use of proxy respondents and selected medical conditions. No correlation between glyphosate exposure and multiple myeloma was reported.

Materials and Methods

Study population - The North American Pooled Project (NAPP) is comprised of three population-based incident case-control studies conducted by the U.S. National Cancer Institute in Kansas, Iowa/Minnesota and Nebraska in the 1980s, and the Cross Canada Study of Pesticides and Health (CCSPH), a population-based incident case-control study that was conducted in Quebec, Ontario, Manitoba, Saskatchewan, Alberta and British Columbia in the early 1990s. All 4 studies aimed to investigate the effects of pesticides and other agricultural exposures on the risk of lymphatic and hematopoietic cancers. The present analysis is restricted to a subset of three NAPP studies conducted in Iowa, Nebraska and Canada (CCSPH) where multiple myeloma (MM) cases were recruited. The study design and data collection in the CCSPH were modeled after the U.S. studies, making the data amenable to pooling. Eligible participants included white men aged 30 years or older in Iowa, white men and women aged 21 years or older in Nebraska, and men aged 19 years or older in Canada. Deceased participants were considered eligible in Iowa and Nebraska, but not in the Canadian study. Proxy respondents were used on behalf of deceased subjects in Iowa and Nebraska, and were permitted in Iowa, Nebraska, and Canada for participants requiring assistance due to illness or disability. Incident MM cases were identified using state and provincial cancer registry records, with the exception of Nebraska and Quebec, where cases were recruited from hospitals. Population-based controls were identified using random digit dialing (all studies), Medicare records and state mortality files (Iowa and Nebraska), health insurance records (Alberta, Saskatchewan, Manitoba and Quebec), telephone listings (Ontario) and voter lists (British Columbia). Cases and controls were frequency-matched to the overall case distribution by age (± 2 years in Nebraska and the CCSPH, ± 5 years in Iowa), vital status and year of death (if applicable), sex (Nebraska) and province of residence (Canada). The participation rates in the MM subset of NAPP studies were modest for Canadian controls (48 %) and higher in Iowa (78 %) and Nebraska (85 %). Participation rates were higher for cases in Canada (58 %), Iowa (84 %) and Nebraska (88 %).

Exposure assessment - A set of *a priori* pesticides to be investigated in this analysis included agents that showed positive (significant or nonsignificant) associations in the earlier U.S. and Canadian studies. Pesticides that met these criteria were 2,4-D, captan, carbaryl, chlordane, DDT, glyphosate, lindane, malathion, methoxychlor, permethrin and the pyrethrins. Self-reported information on pesticide use, farming activities and demographic characteristics were obtained through standardized interviews with participants. Individuals who provided an affirmative answer to general questions about pesticide use or exposure to substances within broad groups (i.e. insecticides, herbicides, fungicides) were subsequently asked more detailed follow-up questions regarding specific agents, including the frequency and duration of exposure. Participants who did not report any pesticide use were classified as unexposed. Among individuals reporting pesticide use, missing information for duration or frequency of exposure was treated as missing or unknown. Information on duration of pesticide use (years) was collected in all studies, whereas frequency information (days per year) was only collected in Canada and Nebraska. The data from Nebraska were excluded from the analysis since the number of exposed cases for pesticides of interest was low and the proportion of missing data was prohibitively high (>40 %).

Exposure metrics - Associations were examined for dichotomous exposure (ever/never pesticide use) and by major chemical classes i.e. phenoxy herbicides, and organochlorine, organophosphate and carbamate insecticides. The duration of exposure was evaluated for each individual pesticide using years of self-reported use. Cumulative exposure was investigated using a composite lifetime days (LD) metric, defined as: LD = years of pesticide use x days/year of pesticide use. Analyses of cumulative exposure were restricted to the Canadian subset of the NAPP data, where sufficient information about both years and days/year was available. For subjects with missing

information for the duration of pesticide use, simple conditional imputation was carried out. Age- and state/province-specific median values for years and days/year were assigned to participants classified as exposed based on the ever/never metric. Imputed values were only assigned if <35 % of exposure duration data were missing among cases, and if the proportions of missing data differed by <20 % between cases and controls.

Statistical analysis - Descriptive analyses were performed on potential confounders identified from the literature including age, province/state of residence, use of a proxy respondent, farming history (ever lived or worked on a farm) and personal medical history. Covariates that were significantly ($p < 0.05$) associated with MM or those that produced meaningful changes (≥5 %) in the OR estimates were retained in the final models. Unconditional logistic regression was used to calculate odds ratios (OR) and 95 % confidence intervals (CI) for pesticide exposure variables with adjustment for age, province/state of residence, use of proxy respondent, and ever being diagnosed with any allergy, hay fever, or rheumatoid arthritis. For all analyses of individual pesticides, the referent population consisted of subjects who did not report any pesticide use, or those who indicated that they had not used that specific agent. Duration of pesticide exposure and cumulative LD were modeled as ordinal variables and linear trends were examined (*p*-trend). Cut-offs for categories were based on the median duration and LD among cases and controls for each pesticide. The use of proxy respondents was also considered as an effect modifier and sensitivity analyses were conducted excluding information provided by proxy respondents All analyses were performed using SAS version 9.3.

Results

The analysis included a total of 587 MM cases and 3,588 controls from Iowa, Nebraska and six Canadian provinces. Female cases ($N = 40$) and female matched controls ($N = 707$) contributed by the Nebraska study were excluded due to the very low prevalence of pesticide use among females. The youngest MM case in the NAPP dataset was 31 years old and, therefore, the controls of an age of 30 years and younger were excluded ($N = 181$) to maintain a comparable distribution of age between cases and controls. The final analysis included 547 male cases and 2,700 male controls.

Among the participants, cases were older than controls. This was expected since a common age-matched control group was used for all cancer sites in the NAPP and the majority of MM cases are typically diagnosed at slightly older ages (>65 years) than non-Hodgkin lymphoma and Hodgkin lymphoma cases. Proxy respondents were used for 35 % of the cases and 28 % of the controls overall.

Associations of demographic characteristics and medical history covariates with MM were modeled using logistic regression, with adjustment for age (in years) and province or state of residence. A history of any type of cancer among first-degree relatives was significantly associated with MM risk. However, having a first-degree relative with any lymphatic or haematopoietic cancer, including MM, was not associated with MM. A number of conditions associated with stimulation of the immune system showed statistically significant inverse associations with MM risk. In this summary only data on glyphosate are reported.

Pesticide exposure (ever/never in the NAPP) - For the "never" use of glyphosate 502 (91.8 %) cases and 2,504 (92.7 %) controls and for the "ever" use of glyphosate 45 (8.2 %) cases and 196 (7.3 %) controls were identified. The adjusted OR was 1.29 (95 % CI 0.9-1.85). When proxy respondents were excluded then the adjusted OR was 1.07 (95 % CI 0.69-1.66).

Pesticide exposure relative to the years of exposure (Iowa and Canada subset of the NAPP) – For no exposure to glyphosate 471 (91.4 %) cases and 1,832 (91.7 %) controls, for up to 3 years of exposure to glyphosate 22 (4.3 %) cases and 87 (4.3 %) controls and for more than 3 years 22 (4.3 %) cases and 80 (4.0 %) controls were identified. The adjusted OR was 1.30 (95 % CI 0.79-2.16) for up to 3 years of exposure and 1.34 (95 % CI 0.80-2.23) for more than 3 years of exposure. The trend was not statistically significant (p = 0.16). When the adjusted OR was taken with exclusion of proxy respondents it was 1.19 (95 % CI 0.67-2.11) for up to 3 years of exposure and 0.95 (95 % CI 0.50-1.83) for more than 3 years exposure. The trend was not statistically significant (p = 0.90).

Pesticide exposure relative to the number of lifetime days (LD) (Canadian subset of the NAPP) - For no exposure to glyphosate 310 (90.6 %) cases and 1,228 (91.0 %) controls, for up to 6 LD of exposure to glyphosate 18 (5.3 %) cases and 65 (4.8 %) controls and for more than 6 LD 14 (4.1 %) cases and 56 (4.2 %) controls were identified. The adjusted OR was 1.35 (95 % CI 0.76-2.40) for up to 6 LD of exposure and 1.11 (95 % CI 0.59-2.07) for more than 6 LD of exposure. The trend was not statistically significant (p = 0.48). When the adjusted OR was taken with exclusion of proxy respondents it was 1.43 (95 % CI 0.76-2.70) for up to 6 LD of exposure and 0.94 (95 % CI 0.44-1.99) for more than 6 LD of exposure. The trend was not statistically significant (p = 0.74).

Discussion and Conclusions

The study authors reported several limitations:
The authors observed several attenuated (and inversed) point estimated for the highest pesticide exposure category, which may be attributed to exposure measurement error, uncontrolled confounding, chance findings or small numbers of cases and controls.
Additionally, recall bias arising from differential reporting of exposure by cases and controls is a concern in case-control studies and may lead to inflated risk estimates. However, the study authors indicate that this pattern of results was not observed in the data.
Despite the increase in the overall sample size resulting from pooling data from the CCSPH and U.S. NCI studies, the numbers of exposed participants were still low for some pesticides, and information for duration or frequency was sparse and not collected in all MM studies. Our ability to investigate the effects of high levels of exposure was further limited, since few participants reported frequent and long- term pesticide use.
Exposure misclassification due to the use of proxy respondents may also influence results. Studies have shown that farmers may be able to recall pesticide use better compared to nonfarmers, and certain types of proxy respondents, such as friends and family members who also work in agriculture, may be more likely to recall the use of certain specific pesticides.
Lastly, it should be recognized that a large number of comparisons were made and some of the effect estimates were based on small numbers of exposed cases and controls. Therefore, it cannot be excluded that some of the observed associations may represent chance findings.
Despite these limitations, this study has several important strengths. The NAPP is one of the largest pooled case-control studies of agricultural exposures and haematopoietic cancers. This analysis was the first to investigate the association between pesticide exposure and MM risk in a pooled sample of Canadian and American participants. Since similar pesticides were used in both Canada and the United States, it was possible to investigate the effects of these exposures in the NAPP overall. Furthermore, similarities in the design of the case-control studies facilitated successful pooling of these datasets, which afforded a larger sample size for more comprehensive and powerful analyses. Specifically, the investigation of different aspects of pesticide exposure, such as duration of use and cumulative lifetime exposure provided an informative and novel addition to this analysis of pesticide use alone. A further advantage of this study was the extensive medical history information that was collected in the Canadian and U.S. studies. This allowed the study authors to take into account the influence of several conditions that result in sustained stimulation of the immune system, such as rheumatoid arthritis, systemic lupus erythematosus, certain viral infections and allergies, which were inversely associated with MM in our data.

No statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Four population-based incident case-control studies (3 US studies and one Canadian study) pooled in the North American Pooled Project (NAPP) aimed to investigate the effects of pesticides and other agricultural exposures on the risk of lymphatic and hematopoietic cancers. The present analysis is restricted to a subset of three NAPP studies (Iowa, Nebraska and Canada) where multiple myeloma (MM) cases were recruited. Self-reported information on pesticide use, farming activities and demographic characteristics was collected and the odds ratios (OR) were calculated for "ever/never" exposure, years of exposure and cumulated lifetime days of exposure to glyphosate with and without exclusion of proxy respondents. The result is that no statistically significant increases in risk of multiple myeloma (MM) associated with self-reported exposure to glyphosate were observed.

This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.

Reliability criteria for epidemiology studies made by the applicant

Publication: Presutti *et al*., 2016	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.	Y	

Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design – epidemiological method followed	Y	Pooled case control studies
Description of population investigated	Y	
Description of exposure circumstances	Y	May be subject to recall bias
Description of results	Y	
Have confounding factors been considered	N	Confounding factors cannot be ruled out
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant for glyphosate risk assessment but reliable with restrictions because it concerns pooled case control studies which are subject to recall bias and in which confounding factors could not be ruled out.		

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007.

Study design and conduct: Reliability moderate – case-control study
Population: Reliability moderate, cases were identified from cancer registry records or recruited from hospitals. Population based controls were identified using random digit dialing, medicare records and state mortality files, health insurance records, telephone listings and voter listings depending on the specific study. Participation rates were quite low for the Canadian study (58% for cases and 48% for controls). Despite the use of pooled data from different studies, the numbers of exposed participants were still low for some pesticides, and information for duration or frequency was sparse and not collected in all MM studies.
Exposure assessment: Reliability moderate, self-reported pesticide use via questionnaire, potential for recall bias although the results do no appear to indicate recall bias.
Outcome assessment: Reliability high : multiple myeloma based on cancer registry records.
Confounder control : Reliability moderate to low: ORs were adjusted for age, province/state of residence, use of proxy respondent and ever being diagnosed with any allergy, hay fever or rheumatoid arthritis. No adjustments were made for other pesticide use. Moreover, no adjustments appears to have been made for cancer among first-degree relatives. Family history is a known risk factor of multiple myeloma and has been not adjusted for in the analysis although the data was available from questionnaires. Radiation and other occupational chemical exposures were not adjusted for.
Statistical methods Reliability high to moderate, appropriate statistical analysis applied, however not all confounders appear to have been taken into account. A good part of the study is that the ORs for all cases and for those where proxy responders are excluded are both reported in the study.
Reporting : Reliability high, key elements of the Material and methods and Results section are reported.

Overall, the reliability of the study is concluded to be moderate (reliable with restrictions). No correlation between glyphosate exposure and multiple myeloma was reported.

B.6.5.18.12. Supporting publications – Sorohan, 2015

Data point:	CA 5.5/037
Report author	Sorahan T.
Report year	2015
Report title	Multiple Myeloma and Glyphosate Use: A Re-Analysis of US Agricultural Health Study (AHS) Data
Document No	doi:10.3390/ijerph120201548 E-ISSN: 1660-4601
Guidelines followed in study	None
Deviations from current test guideline	NA
Previous evaluation	No, study submitted for the purpose of the renewal.
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	Conclusion GRG : Yes/Reliable without restrictions Conclusion AGG: Reliable without restrictions

Full summary of the study according to OECD format

The Relative risks (RRs) for exposed and non-exposed subjects were calculated using Poisson regression; subjects with missing data were not excluded from the main analyses. Using the full dataset adjusted for age and gender the analysis produced a RR of 1.12 (95 % CI 0.50 to 2.49) for ever-use of glyphosate. Additional adjustment for lifestyle factors and use of ten other pesticides had little effect (RR 1.24, 95 % CI 0.52 to 2.94). There were no statistically significant trends for multiple myeloma risks in relation to reported cumulative days (or intensity weighted days) of glyphosate use. The doubling of risk reported previously arose from the use of an unrepresentative restricted dataset and analyses of the full dataset provides no convincing evidence in the AHS for a link between multiple myeloma risk and glyphosate use.

Materials and Methods
The secondary data file which served as a basis for this study was provided by researchers of the Agricultural Health Study (AHS) taking care of the privacy of the participants. AHS researchers supplied an informative description of the file and the file was found to be internally consistent as well as consistent with data descriptions supplied earlier. All subjects gave their informed consent for inclusion before they participated in the AHS study and ethics approval for the original data collection by AHS researchers was obtained from the Institutional Review Board of the National Institutes of Health. This secondary analysis was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the University of Birmingham Science, Technology, Engineering and Mathematics Ethical Review Committee.

Data on lifestyle factors and use of pesticides were collected from 57,311 private and commercial pesticide applicators from Iowa and North Carolina. Previous analyses were carried out on three subsets of data: Set 1 comprised 54,315 applicators excluding those with any cancers diagnosed before enrolment, applicators lost to follow-up, who had missing data for age at enrolment, or who provided no information on whether they had ever used glyphosate. Set 2 comprised 49,211 applicators and further excludes applicators with missing data on level of education, smoking history, or use of alcohol. Set 3 comprised 40,719 applicators and further excludes applicators with missing data on either use or estimated cumulative exposure days for 2,4-D (2,4-dichlorophenoxy acetic acid), alachlor, atrazine, metolachlor, or trifluralin, and missing data on ever-use of benomyl, maneb, paraquat, carbaryl, or diazinon. The objective of this analysis was to examine findings in an as full a dataset as possible and some analyses have also been carried out on a larger fourth set of 55,934 applicators, a category that does not exclude applicators with missing data on ever-use of glyphosate but only applicators with cancers diagnosed before enrolment, applicators lost to follow-up, or who had missing data for age at enrolment.

Poisson regression was used to estimate RRs and 95 % CIs associated with glyphosate exposure metrics, with and without adjustment for other variables. Each variable under analysis was classified into levels or categories. The

analytical approach for the full dataset was to have a "not known/missing" category for each variable so that analyses of all available cases could be maintained. However, it was necessary to ensure that there was at least one case of multiple myeloma in each level of each variable for the regression to successfully calculate RRs. There were no cases of multiple myeloma in those applicators with "unknown use of 2,4-D", such applicators were combined with those reporting "no use" to create a new category of "no claim of use". There were no cases of multiple myeloma in applicators with an "unknown level of education". These applicators were combined with those reporting no education beyond high school. All significance tests were two-tailed and tests for trend (where applicable) were calculated by scoring the levels of a variable and treating the variable as unfactored. All analyses were performed with the EPICURE statistical software, using the double precision DOS version 2.12 of DATAB and AMFIT, dated March 2002.

Results

There were 32 cases of multiple myeloma in Set 1, 26 cases in Set 2, and 22 in Set 3. For the calculation of the RRs there was no adjustment for gender. None of the RRs calculated (RR including statistical adjustment for age at enrolment and RR with additional adjustments) for the three sets is statistically significant and in Set 1, the largest data set, the RR for ever-use of glyphosate is close to unity (RR 1.08, 95 % CI 0.48 to 2.41). The point estimates of risk for the smaller datasets show an approximate doubling of risk irrespective of whether adjustment for other variables is carried out. The largest RR is shown for the fully adjusted model of the smallest dataset (RR 2.79, 95 % CI 0.78 to 9.96).

Estimated risks of multiple myeloma for reported ever-use for the 54,315 applicators in Set 1 (total of 32 cases) were calculated in relation to use of pesticides and other variables (smoking, alcohol consumption, family history of cancer and education). The RR for ever-use of glyphosate, with adjustment for age at enrolment and gender only, is close to unity (RR 1.12, 95 % CI 0.50 to 2.49). The RR for ever-use of glyphosate is little changed with additional adjustment for all 14 other variables (RR 1.24, 95 % CI 0.52 to 2.94).

RR estimates were calculated for multiple myeloma in terms of levels of reported cumulative days of glyphosate use and levels of estimated intensity-weighted exposure days for the 54,315 applicators in Set 1. For each exposure metric three sets of RRs have been calculated: firstly, adjusting for age at enrolment and gender; secondly, with additional adjustment for cigarette smoking, use of alcohol, family history of cancer, and level of education; and thirdly, with additional adjustment for use of ten other pesticides. Two tests for trend were applied to each of these analyses, the first scored the levels of cumulative exposure as 1-4, the second scored the level by mean values. There were no statistically significant trends, but a not statistically significantly elevated RR was shown for the highest category of intensity-weighted exposure days in the fully adjusted model (RR 1.87, 95 % CI 0.67 to 5.27). There were no cases of multiple myeloma in glyphosate users with unknown extent of use.

RR estimates were calculated for multiple myeloma in terms of levels of ever-use of glyphosate, reported cumulative days of glyphosate use, and estimated intensity-weighted exposure days for the 55,934 applicators in Set 4 with a total of 34 cases of multiple myeloma. The risk of multiple myeloma in ever-users of glyphosate was close to unity (RR 1.18, 95 % CI 0.36 to 8.20) and there were no significant trends with either of the two cumulative exposure metrics.

Discussion

This study found no significant trends of multiple myeloma risk with reported cumulative days of glyphosate use and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used. The suspiciously elevated RRs reported previously arose from the use of restricted data sets that, probably by chance, turned out to be unrepresentative. These restrictions would seem to be unnecessary because there is no technical problem in dealing with missing data in Poisson regression. The practice of restricting analyses to subjects with complete data for all variables is, perhaps, a procedure to be carried out with caution as it is clear from this example that such restrictions can lead to misleading findings. It also ignores the fact that findings for missing categories can often be interesting in their own right.

Conclusion

This secondary analysis of AHS data does not support the hypothesis that glyphosate use is a risk factor for multiple myeloma, and suggests that the practice of restricting analyses to subjects with complete data for all variables is perhaps not to be recommended.

3. Assessment and conclusion

Assessment and conclusion by applicant:

In this study the relative risk estimates for exposed and non-exposed applicators were calculated using Poisson regression and subjects with missing data were not excluded from the main analyses. When using the full dataset adjusted for age and gender the analysis produced a RR close to unity for ever-use of glyphosate. Additional adjustment for lifestyle factors and use of ten other pesticides had little effect. This study found no statistically significant trends of multiple myeloma risk with reported cumulative days of glyphosate use and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analyses had adjustment for a few basic variables (age and gender) or adjustment for many other lifestyle factors or pesticide exposures, as long as data on all available pesticide applicators were used. The suspiciously elevated RRs reported previously arose from the use of restricted data sets that, probably by chance, turned out to be unrepresentative.

This publication concerns a secondary analysis of the data from the Agricultural Health Study (AHS) and is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the reliability criteria of a well conducted epidemiology study.

Reliability criteria for epidemiology studies

Publication: Sorahan T., 2015	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines/practices.		
Study completely described and conducted following scientifically acceptable standards	Y	Secondary analysis of the AHS data
Test substance		
Exposure to formulations with only glyphosate as a.s.		
Exposure to formulations with glyphosate combined with other a.s.		
Exposure to various formulations of pesticides	Y	
Study		
Study design – epidemiological method followed	Y	
Description of population investigated	Y	
Description of exposure circumstances	Y	
Description of results	Y	
Statistical analysis	Y	
Overall assessment		
Reliable without restrictions	Y	
Reliable with restrictions		
Not reliable		
This publication concerns a secondary analysis of the data from the agricultural health study and is considered relevant for the risk assessment of glyphosate and reliable without restrictions because it complies with the reliability criteria of a well conducted epidemiology study.		

Assessment and conclusion by RMS:

The study concerns a re-analysis of data from the Agricultural Health Study (AHS). The reliability of this study is already assessed in this RAR for other publications (de Roos, 2005, Andreotti, 2018) and concluded to be high.

Regarding the re-analysis conducted the study was conducted with the aim to understand the conflicting findings of De Roos et al. 2005 study on the risks of multiple myeloma. It is noted in the conflict of interest

that the author received consultancy fees from Monsanto Europe SA/NV although the study author does indicate that the sponsor had no role in the design of the study, analysis or interpretation of the data and that the role of the sponsor in the writing of the manuscript was limited to some stylistic suggestions.

Based on the re-analysis no association between glyphosate exposure and multiple myeloma was found. This is in line with the outcome of the update of the AHS as reported by Andreotti et al. 2018.

B.6.5.18.13. Supporting publications – Alavanja, 2013

Data point:	K-CA 5.5-038
Report author	Alavanja MCR, Ross MK, Bonner MR.
Report year	2013
Report title	Increased cancer burden among pesticide applicators and others due to pesticide exposure
Reference	CA Cancer J Clin 2013; 63: 120-42
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, previously evaluated in the RAR (2015)
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG:** No **Conclusion AGG:** The publication by Alavanja et al. 2013 concerns a review article which sites only one epidemiological study on an association between glyphosate and cancer burden which is already included in the dossier in section B.6.5.18.17. The study does not provide any new information and is therefore considered supplemental.
Additional comment AGG	The provided reference (K-CA 5.5-038) only concerns a correspondence to the article by Alavanja et al. 2013 made by the Executive Director of the Industry Task Force II on 2,4-D. Although the full article by Alavanja et al. 2013 is publicly available online and could be reviewed by the AGG, ***the applicant is requested to submit the full publication to complete the dossier.***

Full summary of the study according to OECD format

This is a review article about numerous pesticides and cancer at a very general level. There is no new information or new epidemiologic analyses for glyphosate or for any other pesticide. The authors conclude that pesticides likely do constitute a cancer risk. For glyphosate, the authors mention non-Hodgkin's lymphoma as being positively associated with glyphosate. The reference cited for this association was Eriksson et al. (2008).

Materials and Methods

Not applicable

Results

No original data.

Discussion

The authors conclude that use of pesticides is likely a cancer risk.

References

Eriksson M, Hardell L, Carlberg M, et al. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. Int J Cancer. 2008; 123:1657-1663.

Assessment and conclusion

Assessment and conclusion by applicant:

There is no new epidemiologic information in this review article and the assessment is conducted at a very high level without due consideration of the validity issues in the various epidemiology studies that are cited for glyphosate or for other individual pesticides. The lead author was the principal investigator for the Agricultural Health Study (AHS) at the time of this publication. That makes it very curious that the authors would cite the case-control study by Eriksson et al. (2008) as evidence for an association between glyphosate and non-Hodgkin's lymphoma (NHL) and not cite the contrary AHS findings for glyphosate and NHL that were published in 2005 (De Roos et al. 2005). In fact, Dr. Alavanja was the senior author on the De Roos et al. (2005) publication. DeRoos et al. (2005) reported on a prospective cohort analysis of glyphosate and NHL (and other cancers) that did not find an association between glyphosate and NHL incidence (RR = 1.1, 95% CI 0.7, 1.9). By virtue of the prospective cohort design of the AHS, the results cannot be affected by recall bias, which is one of the concerns in Eriksson's case control study (see Crump 2020). The most recent glyphosate publication from the AHS also shows no association between glyphosate and NHL with longer follow-up and much larger numbers of NHL cases (Andreotti et al. 2018). So, the review does not provide any new information about glyphosate and it is incomplete in its consideration of the evidence for NHL and glyphosate by failing to cite the De Roos et al. (2005) AHS findings that were published three years before this review.

References

Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509–516.

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Anal 2020; 40(4): 696-704.

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. Environ. Health Perspect. 2005, 113, 49–54.

Eriksson M, Hardell L, Carlberg M, et al. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. Int J Cancer. 2008; 123:1657-1663.

Reliability Criteria: Epidemiology studies

Publication: Alavanja MCR, Ross MK, Bonner MR. Increased cancer burden among pesticide applicators and others due to pesticide exposure. CA Cancer J Clin 2013; 63: 120-42.	**Criteria met? Y/N/?**	**Comments** This is a narrative review with no new information for glyphosate.
Study Design		
Adequate study design given study objectives	No	Not a study. Just a review article.
Appropriate study population to address potential glyphosate-related health outcomes	n/a	n/a (not applicable)
Exposure studied		
Exposure to formulations with glyphosate as a.i.	n/a	
Exposure to formulations with other a.i.	n/a	
Exposure to other farm exposures	n/a	
Study Conduct/analysis		
Adequate description of study population	n/a	
Adequate description of exposure circumstances	n/a	
Comparable participation by groups being compared	n/a	
Information provided by proxy respondents	n/a	
Adequate statistical analysis	n/a	
Adequate consideration of personal confounding factors	n/a	
Adequate consideration of potentially confounding exposures	n/a	
Overall assessment		
Reliable without restrictions	n/a	
Reliable with restrictions	n/a	
Not reliable	n/a	

Assessment and conclusion by RMS:
The study provides a review of available information on pesticide use in general and increased cancer burden. As the study author indicates the review is not exhaustive in its scope or depth and not all available information on glyphosate appears to have been taken into account. The only reference relating to association between glyphosate and cancer burden mentioned is the study by Eriksson et al. (2008) which is reviewed in section B.6.5.18.12. Overall, it is concluded that the study by Alavanja et al. 2013 does not provide any new information.

B.6.5.18.14. *Supporting publications* – Blair and Freeman, 2009

Data point	KCA 5.5-039
Report author	Blair A, Freeman LB.
Report year	2009

Report title	Epidemiologic studies in agricultural populations: Observations and future directions.
Reference	J Agromedicine 2009; 14: 125-131
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, previously evaluated in the RAR (2015)
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Study does not provide any new information not already included in the renewal dossier.

Full summary of the study according to OECD format

This is a general review of the health experience of farmers with some commentary about future directions that the agricultural epidemiology field should consider.

Materials and Methods

Narrative review.

Results

No new results, just a summary of results in the literature.

Discussion

The authors concluded that epidemiologists need to consider the full range of exposures in the farming environment.

Assessment and conclusion

Assessment and conclusion by applicant:

There is no new epidemiologic information in this review article. Glyphosate is not mentioned a single time in the article, so this review has no relevance for evaluating glyphosate.

Reliability Criteria: Epidemiology studies

Publication: Blair A., Freeman LB. Epidemiologic studies in agricultural populations: Observations and future directions. J Agromedicine 2009; 14: 125-131	**Criteria met? Y/N/?**	**Comments** This is a narrative review, not a study. Glyphosate is not mentioned a single time in the article.
Study Design		
Adequate study design given study objectives	No	Not a study. Just a review article.
Appropriate study population to address potential glyphosate-related health outcomes	n/a	n/a (not applicable)
Exposure studied		
Exposure to formulations with glyphosate as a.i.	n/a	
Exposure to formulations with other a.i.	n/a	
Exposure to other farm exposures	n/a	
Study Conduct/analysis		

Publication: Blair A., Freeman LB. Epidemiologic studies in agricultural populations: Observations and future directions. J Agromedicine 2009; 14: 125-131	**Criteria met? Y/N/?**	**Comments** This is a narrative review, not a study. Glyphosate is not mentioned a single time in the article.
Adequate description of study population	n/a	
Adequate description of exposure circumstances	n/a	
Comparable participation by groups being compared	n/a	
Information provided by proxy respondents	n/a	
Adequate statistical analysis	n/a	
Adequate consideration of personal confounding factors	n/a	
Adequate consideration of potentially confounding exposures	n/a	
Overall assessment		
Reliable without restrictions	n/a	
Reliable with restrictions	n/a	
Not reliable	No	Glyphosate is not mentioned a single time in the article.

Assessment and conclusion by RMS:
The study provides a more general overview of epidemiological studies on cancer risk among agricultural populations and provides recommendations on changes in the conduct of future epidemiological studies.
In contrast to the assessment made by the applicant it is noted that glyphosate is mentioned in the report. The study authors states briefly that the AHS cohort showed a potential association between multiple myeloma and glyphosate (no odds ratio provided). The results of the AHS study are already reported in more detail in other publications included in the dossier and therefore the publication by Blair et al. 2009 is not considered to provide any new information.

B.6.5.18.15. Supporting publications – De Roos et al. 2003

Data point	KCA 5.5-040
Report author	DeRoos et al.
Report year	2003
Report title	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men
Reference	Occup Environ Med 2003; 60: 1-9.
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, previously evaluated in the RAR (2015)
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Reliable with restrictions.

Full summary of the study according to OECD format

Background: The purpose of this pooled analysis of 3 US case-control studies was to implement a 2-stage analytic method (hierarchical regression) for dealing with multiple pesticide exposures in studies of non-Hodgkin's lymphoma. **Methods:** During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data resulted in a large enough sample size (650 cases, 1933 controls) to allow analysis of 47 pesticides while simultaneously controlling for potential confounding by other pesticides and adjusting the models' estimates based on a prespecified variance to make them more stable. **Results:** Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A sub-analysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk for some with exposure to increasing numbers. **Conclusion:** Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.

Materials and Methods

Subjects: The three studies included in this pooled analysis had different case ascertainment periods:

- Cases from the Kansas study by Hoar et al. (1986) represented a random sample of cases diagnosed between 1979 and 1981 and selected from the Kansas Cancer Data Service.
- Cases from the study in Iowa and Minnesota by Cantor et al. (1992) were diagnosed between 1981 and 1983 and were identified from the Iowa State Health Registry along with a surveillance system established in Minnesota.

  Cases from the Nebraska study by Zahm et al. (1990) were diagnosed between July 1983 and June 1986 and were identified using the Nebraska Lymphoma Study Group as well as data from area hospitals.

Controls for these studies were randomly selected from population databases (e.g. Medicare, random digit dialling, and state mortality files for deceased cases) and frequency matched to cases on race, sex, age and vital status at time of interview. Data collection regarding pesticide use involved proxies for 40% of cases and 31% of controls. *Exposure assessment:* Cases and controls were interviewed (including next-of-kin when necessary) regarding use of pesticides and/or herbicides as well as other known or suspected risk factors for NHL. Classification as exposed to any specific pesticide required only 1 day of exposure in lifetime. The extent of exposure (number of days or exposure circumstances) was not considered. *Statistical analysis:* Two types of statistical models were used to estimate ORs and 95% CIs: (1) standard logistic regression and (2) hierarchical regression, wherein logistic regression estimates were adjusted in a second stage based on expected similarities of effects within pesticide classes and the presumed a priori carcinogenic probability for specific pesticides as determined by external review bodies. For pesticides like glyphosate that were presumed to have a low probability of being carcinogenic, this second stage adjustment tended to draw positive associations toward the null. All analyses were adjusted for age, study site, and for the use of 46 other pesticides.

Results The final analysis dataset included 650 cases and 1933 controls, after exclusions of individuals (25% of cases and 25% of controls) for whom there was missing information. The state of residence of the cases (67% Iowa/Minnesota, 15% Kansas, 17% Nebraska) was appreciably different than that for controls (46% Iowa/Minnesota, 31% Kansas, 23% Nebraska). These differences were addressed in the statistical models by controlling for study site.

The ORs for the 47 pesticides studied were distributed around the null value of 1.0. According to Crump (2020), 52% of the ORs were > 1, while 48% were less than one. A small number of ORs were greater than 1.3 including organophosphate insecticides coumaphos, fonofos, and diazinon, the organochlorine insecticides chlordane and dieldrin, the insecticide copper acetoarsenite, and the herbicides atrazine, glyphosate, and sodium chlorate. For glyphosate, the OR for the first stage logistic regression was 2.1 (95% CI 1.1, 4.0) and for the second stage hierarchical regression estimate was 1.6 (95% CI 0.9, 2.8).

Discussion

The authors conclude that few pesticides and pesticide combinations were associated with increased NHL risk. Second, the fact that there was a balance between positive and negative associations suggested that the results were not markedly affected by recall bias. Third, although some of the positive results could be due to chance, the hierarchical regression decreased the chance of obtaining false positive results with the possibility of increasing the number of false negative results.

The authors detailed a number of limitations of their study. Foremost was the use of a crude exposure metric of that counted all subjects with any lifetime use as exposed to specific pesticides with no distinctions based on use by the number of years or the number of days per year. Further, the analyses provided no information on the timing of pesticide use in relation to disease onset or in conjunction with the timing of other pesticides used. Lastly, if a study subject had missing information for any one of the 47 pesticides evaluated, that person was excluded from analyses. It is unknown how these exclusions might have affected the results of this study.

References

Cantor KP, Blair A, Everett G, et al. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 1992; 52:2447–2455.

Hoar SK, Blair A, Holmes FF, et al. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA,1986;256:1141–1147.

Zahm SH, Weisenburger DD, Babbitt PA, et al. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1990; 1:349–356.

Assessment and conclusion by applicant:

The pooled analysis by DeRoos and colleagues (2003) considers cases and controls from a time period soon after glyphosate's initial US approval in 1974. Specifically, 67% of cases were identified between 1980-83, 16% of cases were identified during the period 1979-81, and 17% of the cases dated from the period 1983-86. The actual date of first use for cases was not considered in the analysis, but presumably the interval between first glyphosate use and NHL diagnosis was short for most cases. Usually, to cause cancer over short time intervals, one thinks of highly cytotoxic exposures at high doses or highly immunosuppressive exposures at high doses. Glyphosate fits neither profile. Given the low internal doses that were seen in biomonitoring studies of farmers (median 10^{-4} mg/kg; maximum observed 10^{-3} mg/kg) (Acquavella et al. 2004), the low toxicity of glyphosate (USEPA 2017), and the somewhat limited use of glyphosate in its early post-approval days as farmers adjusted from selective herbicides to glyphosate (a non-selective herbicide), it seems unlikely that the exposure scenario considered in this study is biologically plausible. It hinders evaluation further that the authors did not provide information on the frequency of use of glyphosate. That would help with the evaluation of the face validity for the glyphosate findings.

The authors appear to have conducted a very sophisticated statistical analysis and, unlike other case-control studies in the glyphosate literature, there wasn't a marked skew toward positive results across all pesticides. Crump found 52% of ORs were > 1.0 for all pesticide evaluations and 53% for the evaluations of pesticides other than glyphosate (Crump 2020). De Roos et al. (2005) were

forthcoming about the limitations of their study mentioning the crude binary exposure metric, not considering the interval between exposure and outcome (what epidemiologists call the induction-latent period (see Rothman 1978)), and the exclusion of 25% of subjects due to incomplete (missing) or uncertain pesticide information (e.g., marking the questionnaire "don't know" for a specific pesticide). Another limitation that should have been given more consideration was the large amount of exposure information that was provided by proxy respondents and the imbalance in proxy respondents for cases (40%) and controls (31%).

Recently Parwa et al. (2019) conducted a pooled reanalysis of the NHL data from the McDuffie et al. (2001) and DeRoos et al. (2003) studies. The reanalysis sought to evaluate associations for glyphosate use and NHL implementing more extensive control of confounding factors than in the original publications and considering the impact of excluding pesticide information provided by next-of-kin or proxy respondents. The pooled OR for NHL overall for any use of glyphosate was 1.4 (95% CI 1.1, 1.8). After adjustment for other pesticides, the OR was reduced to 1.1 (95% CI 0.8, 1.5) and the OR was reduced further to 0.95 (95% CI 0.7, 1.3) after excluding data from proxy respondents [supplemental table 1 in the article]. It's hard to square the results of this reanalysis with the results reported by De Roos et al. (2003). Parwa et al. (2019) seem to have retained several hundred more cases and controls from the US studies then were retained by De Roos et al. (2003). That fact, plus the exclusion of proxy responses appears to have resulted in OR estimates for any glyphosate exposure (the DeRoos et al. 2003 exposure metric) that are decidedly lower than those reported by De Roos et al. (2003). This would suggest that selection bias due to exclusions in the analysis and differences in the results for proxy versus self-respondents contributed to the higher ORs seen in De Roos et al. (2003) compared to the recently published analyses by Parwa et al. (2019). Another reanalysis specific to purpose would be needed to sort out the apparent differences in the analyses by De Roos et al. (2003) and Parwa et al. (2019).

In conclusion, the studies pooled by De Roos et al. (2003) were initially conducted to study phenoxyacetic acids that were approved in the 1940s. The time period of case ascertainment in the pooled analysis provides for a reasonable induction-latent period for pesticides introduced into agriculture in the 1940s and 1950s. For glyphosate, approved in 1974, the interval between approval (really, we would be interested in the actual date of first use by study subjects, which would be later) and case ascertainment was probably too short to be credible for a causal relationship. Such a short interval does not fit what is known about the exposure circumstances or toxicology (USEPA 2017) of glyphosate. It seems likely, therefore, that there were some issues of bias that were not apparent in the De Roos et al. (2003) study that resulted in spurious elevated ORs for glyphosate. The analysis by Parwa et al. (2019) suggests that exclusions for missing or incomplete data and proxy responses may explain the elevated ORs for glyphosate in De Roos et al. (2003). For all these reasons, the elevated ORs for glyphosate reported by De Roos et al. (2003) are not considered to be credible.

References

Acquavella JF, Alexander BH, Mandel JS, et al. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. Environ Health Perspect 2004; 112:321-326.

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Anal 2020; 40(4): 696-704.

De Roos, A. J., Zahm, S. H., Cantor, K. P., et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine 2003; 60:1–9.

Johnson RA, Mandel JS, Gibson RW, et al. Data on Prior Pesticide Use Collected from Self-and Proxy Respondents. Epidemiology 1993; 4:157-164.

Lee WJ, Colt JS, Heineman EF, et al. Agricultural pesticide use and risk of glioma in Nebraska, United States. Occup Environ Med. 2005; 62:786–792.

McDuffie HH, Pahwa P, McLaughlin JR, et al. Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men: Cross-Canada Study of Pesticides and Health. Cancer Epidemiol Biomark Prev 2001; 10:1155–1163

Parwa M, Freeman LB, Spinelli JJ, et al. Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project. Scand J Work Environ Hlth. 2019;45(6):600–609.

Rothman KJ. Induction and latent periods. Amer J Epidemiol 1978: 114(2): 253-9.

USEPA Office of Pesticide Programs. Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential, December 2017.

Reliability Criteria: Epidemiology studies by the applicant

Publication: De Roos AJ, Zahm SH, Cantor KP, et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine 2003; 60:1–9.	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	No	High proportion of proxy respondents: 40% cases, 31% controls.
Appropriate study population to address potential glyphosate-related health outcomes	No	Insufficient induction-latent period for glyphosate.
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	Due to proxy respondents
Comparable participation by groups being compared	Yes	Though more proxies for cases than controls
Information provided by proxy respondents	Yes	
Adequate statistical analysis	No	Did not consider proxy respondents. A large proportion of subjects excluded due to any missing covariate information.
Adequate consideration of personal confounding factors	Yes	
Adequate consideration of potentially confounding exposures	No	Reanalysis in 2019 gave much different adjusted results
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability moderate, case-control study.
Population : Reliability high – cases obtained from hospitals and Health/Cancer Registries, controls randomly selected from same geographical areas as the cases and matched to race, sex, age and vital status at time of the interview.
Exposure assessment : Reliability moderate, exposure assessment based on interview, fairly high number of proxy responders.
Outcome assessment : Reliability moderate, based on medical records (non-confirmed).
Confounder control : Reliability high, potential for confounding checked for age, first degree relative with haematopoietic cancer, education and smoking. ORs were adjusted for use of other pesticides.
Statistical methods : Reliability high, appropriate statistical methods are used, adequate control for confounders

Reporting : Reliability high, key elements of the Materials and Methods and the Results section are reported in sufficient detail.

One of the limitations reported by the applicant is that the cases were reported between 1979 and 1986 and that since glyphosate was first approved in 1974 the case ascertainment was probably too short to be credible for a causal relationship. The RMS however notes that based on public literature information it seems that the latency period for Non-Hodgkin lymphoma is largely unknown. In patients with Hodgkin's disease treated with chemotherapy/radiotherapy it seems the latency period can be as short as two years but also as long as 15 years (median 5-6 years)[1]. Other studies seem to report longer latency periods of up to 20 years[2]. Since there is uncertainty regarding the latency period of NHL the RMS does not consider that the somewhat short time between approval and case identification can lower the reliability of the study.

Overall, the study is concluded to be reliable with restrictions.

The data from this study was pooled by Pahwa with a Canadian case-control study. In contrast, to the de Roos, 2003 study this pooled analysis did not find an association between NHL with ever use of glyphosate with an adjusted OR of 1.13 (95% CI 0.84-1.51). One main difference between these two studies is that the study by Pahwa et al. did not exclude subjects with missing pesticide use data and used slightly different covariates in the analysis.

Cited reference:

[1] Weisenburger D.D. Pathological classification of non-hodgkin's lymphoma for epidemiological studies. Cancer Research Suppl. 1992, 52 : 5456s-5464s

[2] Wheeler et al. Spatial-temperol analysis of non-Hodgkin lymphoma in the NCI-SEER NHL case control study. Environmental Health 2011, 10: 63

B.6.5.18.16. Supporting publications – De Roos et al. 2005

Data point	KCA 5.5-041
Report author	DeRoos et al.
Report year	2005
Report title	Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 113:49-54.
Reference	Environ Health Perspect 2005; 113: 49-54
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, RAR (2015), study concluded to be reliable without restrictions
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Reliable with restrictions Conclusion AGG: Study reliable with the exception of low number of cases for multiple myeloma.

Full summary of the study according to OECD format

Background: The authors evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. **Methods:** Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrollment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were

men. In this analysis, exposure was evaluated by three metrics: 1) any lifetime use; 2) cumulative lifetime days of use; and 3) intensity-weighted cumulative exposure days. **Statistical analysis:** Poisson regression was used to estimate relative risks (RRs) and 95% confidence intervals (CIs) between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. RRs were adjusted for demographic and lifestyle factors, including age, education, cigarette smoking, alcohol consumption, family history of cancer in a first-degree relative, and state of residence. Potential confounding from exposure to other pesticides was addressed by including in the Poisson models the five pesticides most highly associated with glyphosate exposure: 2,4-D, alachlor, atrazine, metolachlor, and trifluralin. **Results:** Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma that should be followed up as more cases occur in the AHS. **Conclusion:** Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including for less common cancers.

[Note, this study has been updated in a 2018 publication. See Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509–516.]

Materials and Methods

Subject recruitment: The AHS is a prospective cohort study in Iowa and North Carolina, which includes 57,311 private and commercial applicators who were licensed to apply restricted-use pesticides at the time of enrollment. Recruitment of the applicators occurred between 1993 and 1997 (see Alavanja et al. 1996 for details).

Outcome assessment: Cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and the National Death Index (National Center for Health Statistics 1999) to ascertain vital status. Incident cancers were identified for the time period from the date of enrollment until 31 December 2001 and were coded according to the International Classification of Diseases, 9th Revision (WHO 1977). If cohort members had moved from the state, they were censored in the year they left. The median time of follow-up was 6.7 years.

Exposure assessment: Using a self-administered enrollment questionnaire, the authors collected comprehensive-use data on 22 pesticides, ever/never use information for 28 additional pesticides, and general information on pesticide application methods, personal protective equipment, pesticide mixing, and equipment repair. Data were also collected on basic demographic and lifestyle factors. Applicators who completed this questionnaire were given a self-administered take-home questionnaire, which contained additional questions on occupational exposures and lifestyle factors.

The authors developed three glyphosate exposure metrics: 1) any lifetime use; 2) cumulative lifetime days of use; and 3) intensity-weighted cumulative exposure days.

Statistical analyses: Poisson regression analyses were carried out for all cancers combined and specific cancer sites to estimate rate ratios (RRs) and 95% confidence intervals (CIs) associated with glyphosate exposure metrics. For each exposure metric, RRs were adjusted for demographic and lifestyle factors, including age, education, cigarette smoking, alcohol consumption, family history of cancer in a first-degree relative, and state of residence. Potential confounding from exposure to other pesticides was addressed by including in the Poisson models the five pesticides most highly associated with glyphosate exposure: 2,4-D, alachlor, atrazine, metolachlor, and trifluralin.

Results

The analysis included 2,088 cancers and the fully adjusted RR for all cancers and any glyphosate exposure was 1.0 (95% CI 0.9, 1.2). There were no significantly elevated RRs for any cancer site. The analysis also included 190 lymphopoietic cancers and the RR for glyphosate exposure and all lymphopoietic cancers was 1.1 (95% CI 0.8, 1.6). Among the lymphopoietic cancer subtypes, RRs were: 1.1 (95% CI 0.7, 1.9) for non-Hodgkin's lymphoma (NHL), 1.0 (95% CI 0.5, 1.9) for leukemia, and 2.6 (95% CI 0.7, 9.4) for multiple myeloma. Analyses by days of use and intensity weighted days of use did not reveal any significant trends for individual cancers, though the analyses for multiple myeloma tended to find higher RRs in the higher cumulative exposure category, although the number of cases was small (n=19 in adjusted analyses of exposure-day metrics). Small numbers of multiple myeloma cases precluded trend analyses that were statistically precise.

Table 2. Association of glyphosate exposure (ever/never used) with common cancers[a] among AHS applicators.

Cancer site	Total no. of cancers[c]	Ever used glyphosate (% of total)	RR (95% CI)[b] Effect estimates adjusted for age (n = 54,315)[d]	RR (95% CI)[b] Adjusted for age, demographic and lifestyle factors, and other pesticides[d]
All cancers	2,088	73.6	1.0 (0.9–1.1)	1.0 (0.9–1.2)
Lung	204	72.1	1.0 (0.7–1.3)	0.9 (0.6–1.3)
Oral cavity	59	76.3	1.1 (0.6–2.0)	1.0 (0.5–1.8)
Colon	174	75.3	1.1 (0.8–1.6)	1.4 (0.8–2.2)[e]
Rectum	76	77.6	1.2 (0.7–2.1)	1.3 (0.7–2.3)
Pancreas	38	76.3	1.2 (0.6–2.5)	0.7 (0.3–2.0)[e]
Kidney	63	73.0	1.0 (0.6–1.7)	1.6 (0.7–3.8)[e]
Bladder	79	76.0	1.2 (0.7–2.0)	1.5 (0.7–3.2)[e]
Prostate	825	72.5	1.0 (0.8–1.1)	1.1 (0.9–1.3)
Melanoma	75	84.0	1.8 (1.0–3.4)	1.6 (0.8–3.0)
All lymphohematopoietic cancers	190	75.3	1.1 (0.8–1.5)	1.1 (0.8–1.6)
NHL	92	77.2	1.2 (0.7–1.9)	1.1 (0.7–1.9)
Leukemia	57	75.4	1.1 (0.6–2.0)	1.0 (0.5–1.9)
Multiple myeloma	32	75.0	1.1 (0.5–2.4)	2.6 (0.7–9.4)[f]

[a]Cancers for which at least 30 subjects had sufficient information for inclusion in age-adjusted analyses. [b]RRs and 95% CIs from Poisson regression models. [c]Frequencies among subjects included in age-adjusted analyses. [d]Numbers of subjects in these analyses are lower than in age-adjusted analyses because of missing observations for some covariates (models adjusted for demographic and lifestyle factors include 49,211 subjects; models additionally adjusted for other pesticides include 40,719 subjects). [e]Estimates adjusted for other pesticides are shown because inclusion of other pesticide variables in the model changed the effect estimate for glyphosate by at least 20%. [f]The estimate for myeloma was not confounded by other pesticides according to our change-in-estimate rule of ≥ 20%; however, the fully adjusted estimate is shown for the purpose of comparison with state-specific estimates (in the text), which were confounded by other pesticides and required adjustment.

Discussion

The authors concluded that there was no association between glyphosate exposure and all cancer incidence or for most of the specific cancer subtypes that were evaluated across exposure metrics. The one potential association in their analyses was between multiple myeloma and glyphosate exposure, based on a small number of cases. The association observed was with ever use of glyphosate and cumulative exposure days (a combination of duration and frequency), but not with intensity of exposure. The authors concluded that this should be followed up as more cases occur in the AHS.

References

Alavanja MC, Sandler DP, McMaster SB, et al. The Agricultural Health Study. Environ Health Perspect 1996; 104:362–369.

Assessment and conclusion

Assessment and conclusion by applicant:

DeRoos et al. 2005 was the first prospective cohort study publication concerning glyphosate exposure. As such, it obviated any concern about case recall bias that was a frequent criticism of the case control studies that are the biggest part of the glyphosate literature. The prospective cohort study was well conducted and featured sophisticated analyses that addressed confounding by personal factors and exposure to other pesticides. The study included a large number of glyphosate

users and many in the parent Agricultural Health Study (AHS) cohort have used glyphosate for decades. DeRoos et al. (2005) has been superseded by an updated glyphosate AHS glyphosate evaluation that includes 3.5 times as many cancer cases, follow-up extended 12 years, and sophisticated methods for addressing missing covariate data (Andreotti et al. 2018).

The one finding of potential concern in the DeRoos et al. (2005) study was the elevated RR for multiple myeloma in the fully adjusted model (RR = 2.6, 95% CI 0.7, 9.4). This finding was markedly different than the multiple myeloma finding in the age adjusted model (RR = 1.1, 95% CI 0.5, 2.4) and there were indications in the fully adjusted cumulative exposure analyses of elevated multiple myeloma RRs in the higher exposure categories, though trend statistics were not significant. As DeRoos et al. (2005) noted, a noteworthy difference between the age adjusted analyses and the fully adjusted analyses was the exclusion of more than 14,000 cohort members (~ 25% of the cohort) who had some missing covariate data. The exclusions included 13 multiple myeloma cases (42% of the MM cases) and these excluded cases were disproportionally from the unexposed category (Acquavella et al. 2016). Sorahan (2015) illustrated the bias from these exclusions in a reanalysis of the data from the DeRoos et al. (2005) publication by including those with missing covariate data in a fully adjusted analysis. Andreotti et al. (2018), in a successor publication to DeRoos et al. (2005), used current recommended analysis techniques to deal with missing data, including imputation. For MM, they found that there was no relationship with glyphosate during the overall study period or during the DeRoos et al. (2005) study period.

The DeRoos et al. publication was the highest quality glyphosate epidemiologic study of its time. As a prospective cohort study, there was no concern about recall bias in the pesticide exposure assessment and the authors used sophisticated analytic methods to control for confounding by personal factors and other pesticide exposures. It provided evidence that NHL was not associated with glyphosate exposure. With the elevated RR for multiple myeloma in the fully adjusted analyses now resolved, the study is best interpreted as providing evidence that glyphosate was not related to cancer incidence among AHS cohort members. It is largely of historical interest now with the publication by Andreotti et al. (2018) that greatly extends the AHS evaluation of glyphosate in years of follow-up and in analytic scope.

References

Acquavella J, Garabrant D, Marsh G, et al. Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma. Critical Rev Toxicol 2016:46(S1):28-43.

Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509–516.

De Roos AJ, Blair A, Rusiecki JA, et al. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 113:49-54.

Sorahan T. Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. Int J Environ Res Public Health 2015; 12:1548–1559.

Reliability Criteria: Epidemiology studies made by applicant

Publication: De Roos AJ, Blair A, Rusiecki JA, et al. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 113:49-54.	**Criteria met? Y/N/?**	**Comments** See updated analysis of this cohort: Andreotti et al. J Natl Cancer Inst. 2018; 110(5): 509–516.
Study Design		
Adequate study design given study objectives	Yes	

Publication: De Roos AJ, Blair A, Rusiecki JA, et al. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 113:49-54.	**Criteria met? Y/N/?**	**Comments** See updated analysis of this cohort: Andreotti et al. J Natl Cancer Inst. 2018; 110(5): 509–516.
Appropriate study population to address potential glyphosate-related health outcomes	Yes	
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	No	
Adequate statistical analysis	Yes	With exception of multiple myeloma analysis, subsequently corrected by Andreotti et al. 2018.
Adequate consideration of personal confounding factors	Yes	
Adequate consideration of potentially confounding exposures	Yes	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	Yes	All but the multiple myeloma results. But, see Andreotti et al for updated results for multiple myeloma, etc.
Not reliable	No	

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007.

Study design and conduct: Reliability high - prospective cohort study, well described study design
Population: Reliability high - all individuals from Iowa and North Carolina seeking a pesticide license were invited to participate. Sufficient sample size with the exception for multiple myeloma. Characteristics of the study population well described.
Exposure assessment: Reliability moderate - exposure assessment via questionnaire with comprehensive-use data for 22 pesticides and ever/never use for 28 additional pesticides. No biomonitoring or external exposure assessment.
Outcome assessment: Reliability high – cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and the Nation Death Index to ascertain vital status.
Confounder control : Reliability high: adequate control for demographic and lifestyle factors, including age at enrollment, education, pack-years of cigarette smoking, alcohol consumption and family history of cancer. Potential confounding from exposure to other pesticides was explored by adjusting for the five pesticides most highly associated with glyphosate cumulative exposure days.
Statistical methods : Reliability high : statistical method appropriate to the study design, methods used to control for confounding.
Reporting : Reliability high: methods and results are adequately described.

Overall, the study did not show an association between glyphosate and cancer incidences, with the possible exception of multiple myeloma which was however not significant and based on a low number of cases. The authors recommended that this should be followed up as more cases occur in the AHS. The follow up study by Andreotti et al. (2018), did not find an association between glyphosate exposure and multiple myeloma.

B.6.5.18.17. Supporting publications – Eriksson, 2008

Data point	KCA 5.5-042
Report author	Eriksson et al.
Report year	2008
Report title	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis
Reference	International J Cancer 2008; 123: 1657-1663
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previously evaluated	Yes, RAR 2015, study concluded to be not reliable
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Study of low reliability (see assessment by RMS at the end of the study summary).

Full summary of the study according to OECD format

The authors conducted a population-based case–control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL) in Sweden. **Methods:** Male and female cases aged 18–74 years were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91%) cases and 1016 (92%) controls participated. **Statistical analysis:** Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals. **Results:** Exposure to herbicides gave an OR of 1.72, 95% CI 1.18–2.51. Regarding phenoxyacetic acids, highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27–6.22. Exposure to glyphosate was associated with an OR 2.02, 95% CI 1.10–3.7 in a univariate analysis and an OR of 1.51 (95% CI 0.77, 2.94) in a multivariate analysis. **Conclusions:** The authors considered that their results confirmed an earlier association between exposure to phenoxyacetic acids and NHL and that the evidence for an association between glyphosate and NHL was considerably strengthened.

Materials and Methods

Subject selection: Cases were identified through physicians who diagnosed and treated NHL, and all cases were verified histologically. Controls were randomly chosen from population registries in the same health service regions as the cases, and were frequency matched in 10-year age and sex groups. A total of 910 NHL cases and 1016 controls were included in the analyses.

Exposure assessment: All subjects received a mailed questionnaire focusing on total work history and exposure to pesticides, solvents and other chemicals. For all pesticides, the number of years, number of days per year and length of exposure per day were questioned. The questionnaire also included questions on smoking habits, medications, leisure time activities and proximity of the home to certain industrial installations but data on these factors were not included in the article.

Statistical analyses: Unconditional logistic regression was used to calculate ORs and 95% CIs, adjusted for age, sex, and year of diagnosis. In analyses for individual pesticides, the unexposed category consisted of subjects that were unexposed to all pesticides. When multivariate analyses were done for specific pesticides, other specific pesticides were included in the models if they had a statistically significant increased OR (presumably in univariate analyses), or with an OR > 1.50 and at least 10 exposed subjects.

Results

Analyses for individual pesticides showed elevated ORs for nearly every agent, although not in every analysis by NHL subtype or category of duration of exposure. The univariate[3] OR for glyphosate was 2.02 (95%-CI 1.10, 3.71) and the multivariate OR was appreciably lower (OR = 1.51, 95%-CI 0.77, 2.94), indicative of confounding of the glyphosate/NHL association. The association between glyphosate and NHL was stratified by median days of use for controls (≤ 10 days, > 10 days). ORs were 1.69 (95%-CI 0.70, 4.07) for 10 days or less and 2.36 (95%-CI 1.04, 5.37) for more than 10 days of use. The authors also provided analyses with latency periods of ≤ 10 years and > 10 years. For glyphosate, the ORs were 1.11 (95%-CI 0.24, 5.08) for ≤ 10 years and 2.26 (95%-CI 1.16, 4.40) for > 10 years. These days of use and latency ORs were adjusted for age, sex and year of diagnosis, but apparently not for other exposures.

Discussion

The authors concluded that the results of this study for phenoxyacetic acid herbicides confirmed the results of their earlier pesticide case-control study and that the indication of an association between glyphosate and NHL in a previous study has been considerably strengthened (presumably referring to Hardell et al. 2002).

References

Hardell L, Eriksson M, Nordstrom M. 2002. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leukemia and Lymphoma. 43:1043–1049.

Assessment and conclusion by applicant:

The NHL case-control study by Eriksson et al. (2008) is similar in very many respects to the previous Swedish NHL case-control study by Hardell, Eriksson, and Nordstrom (2002, B.6.5.18.16). The most noticeable similarity is that in both studies virtually every OR is elevated across pesticide classes and for individual pesticides. This strongly selects that recall bias is distorting the results. Crump conducted an analysis to try to discern whether recall bias and/or selection bias were operative in the case-control studies that are part of the glyphosate epidemiologic literature. His thesis was that since the studies that included glyphosate also included many other pesticides, one could get a measure of whether the methodology was biased by assessing the proportions of non-glyphosate ORs that were greater than the null value of 1.0. If the methodology was unbiased, the proportion of ORs greater than 1.0 for non-glyphosate exposures should be approximately 50%. For Eriksson et al. (2008), the percent of non-glyphosate ORs that were greater than 1.0 was 90%. As a result, Crump (2020) concluded that studies like Eriksson et al. (2008), with such a marked skew toward positive results for non-glyphosate exposures, are not reliable for determining validly exposure-disease relationships for glyphosate.

[3] The authors use of the term univariate meant adjusted for age, sex, and year of diagnosis or enrollment. It was univariate in the sense of not controlling for other exposures.

Another important limitation is the limited extent of the multivariate analyses for glyphosate and other individual pesticides. The authors presented univariate and multivariate results for any glyphosate exposure. The multivariate OR (OR = 1.5, 95% CI 0.8, 4.6) was appreciably lower than the univariate OR (OR = 2.0, 95% CI 1.1, 3.7), indicative of confounding of the glyphosate/NHL association. Yet, multivariate adjustment was not carried over to analysis of glyphosate by days of use or in analyses that considered latency. One would presume that those latter analyses are confounded.

Additional limitations have been pointed out, including potential selection bias (case participation 81%, control participation 65%) that results primarily from the markedly lesser participation of controls in the parent case control study (see Chang and Delzell 2016, Table 1). Acquavella et al. (2016) also pointed out that in Eriksson's analyses the unexposed category consisted of subjects that were unexposed to all pesticides. This definition of unexposed subjects is a misapplication of case-control theory which assumes that comparisons of exposed and unexposed subjects can be implemented such that cases and controls are comparable on all factors except for the exposure under study (viz. the counterfactual). Since those who use glyphosate also use other pesticides, the unexposed group should include individuals with those other exposures (as would be the case in cohort analyses like those of the Agricultural Health Study (see Andreotti et al. 2018)). According to accepted case-control theory (Rothman et al. 2008), the validity of case-control studies depends on accurately estimating the exposure prevalence in the population that gave rise to the cases. Clearly, those who used pesticides other than glyphosate are part of the population that gave rise to the cases in this study. Exposure prevalence cannot be estimated accurately by excluding from the reference group those with pesticide exposures other than the exposure of interest. This practice distorts exposure prevalences and can bias OR estimates (see a numerical illustration in Acquavella et al. 2016). The exclusions also preclude being able to adequately control for the potential confounding of other pesticide exposures (Chang and Delzell 2016).

In conclusion, the glyphosate results in this study are unreliable due to likely recall bias and indications of residual confounding due to the failure to conduct adequate multivariate analyses. There are also concerns about selection bias and analytic selection bias.

References

Acquavella J, Garabrant D, Marsh G, et al. Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma. Critical Rev Toxicol 2016:46(S1):28-43.

Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509–516.

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Anal 2020; 40(4): 696-704.

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; 43:1043-1049.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Eriksson M, Hardell L, Carlberg M, et al. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. Int J Cancer. 2008; 123:1657-1663.	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	No	Strong evidence of recall bias. Limited statistical analysis.
Appropriate study population to address potential glyphosate-related health outcomes	Yes	
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	Yes	
Comparable participation by groups being compared	No	Cases 81%, controls 65%
Information provided by proxy respondents	No	
Adequate statistical analysis	No	Did not address confounding adequately. Analytic selection bias likely.
Adequate consideration of personal confounding factors	Yes	
Adequate consideration of potentially confounding exposures	No	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	Clear from the analysis by Crump (2020) that the methodology was biased by recall bias or selection bias. Also, inadequate control for confounding.

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability moderate to low - case-control study with potential for recall bias, a potential selection bias may results from a lower percentage of participation in the study among invited controls (65%) than among invited cases (81%).
Population: Reliability moderate: cases were referred by their physician, referral bias have may have occurred.
Exposure assessment: Reliability moderate to low, questionnaire answered by subjects. Potential for recall bias which is evidenced by the high odds ratios for virtually all pesticide classes evaluated. Furthermore, the article mentions that interviewers collected information by telephone if important information is missing. It is not mentioned what in meant by 'important'. It is noted that that interviewers received specialized training, which improved the quality of exposure assessment.
Outcome assessment: Reliability high, cases confirmed by physicians

Confounder control : Reliability low, only adjustments were made for age, sex and year of diagnosis or enrolment. Lifestyle factors such as smoking and alcohol use were not included. Moreover, no adjustment was made for history of NHL in first-degree relative nor were adjustments made for use of other pesticides. Considering that the univariate analysis was made compared to an unexposed group to all pesticides it is likely that differences in socio-economic status between the two groups occurred.
Statistical methods : Reliability low, in the univariate analysis different pesticides were analysed separately and the unexposed category consisted of subjects that were unexposed to all included pesticides which can be expected to lead to differences between the groups based on other covariates. It would have been more accurate to compare to groups that used other pesticides. In addition, no multivariate analysis was conducted on the analysis of glyphosate exposure by days or by latency period.
Reporting: Reliable moderate, sufficient information is provided on the key elements of the material and methods section and the results. However, descriptive statistics were not provided.

Due to the potential for recall bias, lack of adjustment for confounders and the limitation noted in the statistical analysis it is agreed that this study is of low reliability.

B.6.5.18.18. Supporting publications – Freeman, 2009

Data point	K-CA 5.5-043
Report author	Freeman LB
Report year	2009
Report title	Evaluation of agricultural exposures: The agricultural health study and the agricultural cohort consortium
Reference	Reviews Environ Health 2009: 24(4): 311-318
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previously evaluated	Yes, RAR 2015
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Study is a review study that does not provide any new information not already included in the RAR.

Full summary of the study according to OECD format

This is a review article by the current principal investigator of the Agricultural Health Study (AHS). It provides a high-level summary of some of the work that has been completed in the AHS as of the date of this publication and some of the things that are anticipated in the future – especially analyses based on a consortium of agricultural cohorts from various countries. Glyphosate is only mentioned once in a table with the indication that there have been no cancer risks associated with glyphosate exposure in the AHS. This conclusion is based on the De Roos et al. (2005) publication.

References

De Roos AJ, Zahm SH, Cantor KP, et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occup Environ Med. 2003; 60: 1-9.

Materials and Methods

Narrative review.

Results

Summary of AHS activities as of the date of publication.

Discussion

The author gives a perspective on future research activities and on the nascent formative activities for the multi-country agricultural cohort consortium.

3. Assessment and conclusion

Assessment and conclusion by applicant:

There is no new information in this review article that is relevant to the evaluation of glyphosate epidemiologic research.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Freeman LB. Evaluation of agricultural exposures: The Agricultural Health Study and the Agricultural Cohort Consortium. Reviews Environ Health 2009: 24(4): 311-318.	**Criteria met? Y/N/?**	**Comments** This is a narrative review with no new information for glyphosate.
Study Design		
Adequate study design given study objectives	No	Not a study. Just a review article.
Appropriate study population to address potential glyphosate-related health outcomes	n/a	n/a (not applicable)
Exposure studied		
Exposure to formulations with glyphosate as a.i.	n/a	
Exposure to formulations with other a.i.	n/a	
Exposure to other farm exposures	n/a	
Study Conduct/analysis		
Adequate description of study population	n/a	
Adequate description of exposure circumstances	n/a	
Comparable participation by groups being compared	n/a	
Information provided by proxy respondents	n/a	
Adequate statistical analysis	n/a	
Adequate consideration of personal confounding factors	n/a	

Publication: Freeman LB. Evaluation of agricultural exposures: The Agricultural Health Study and the Agricultural Cohort Consortium. Reviews Environ Health 2009: 24(4): 311-318.	**Criteria met? Y/N/?**	**Comments** This is a narrative review with no new information for glyphosate.
Adequate consideration of potentially confounding exposures	n/a	
Overall assessment		
Reliable without restrictions	n/a	
Reliable with restrictions	n/a	
Not reliable	n/a	

Assessment and conclusion by RMS:
The reference provides an overview of the Agricultural Health Survey. The only reference made to glyphosate is a reference to the study by De Roos, 2005 (evaluated in B.6.5.18.11). No new information is provided.

B.6.5.18.19. Supporting publications – Fritschi, 2005

Data point	KCA 5.5-044
Report author	Fritschi L, et al.
Report year	2005
Report title	Occupational Exposure to Pesticides and Risk of Non-Hodgkin's Lymphoma
Reference	Amer J Epidemiol 2005; 162: 849-857
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previously evalauted	Yes, RAR 2015
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Study does not provide information on glyphosate specifically, but only on combined pesticide exposure.

Full summary of the study according to OECD format

The authors undertook a population-based non-Hodgkin's lymphoma (NHL) case-control study in New South Wales, Australia. **Methods:** NHL cases (n = 694) during the years 2000–2001 were identified through the Central Cancer Registry for New South Wales. Controls (n = 694) were selected from electoral roles. Cases and controls were interviewed with respect to their occupational histories and an industrial hygienist assigned pesticide exposures based on professional judgment and a crop-pesticide matrix. **Statistical analysis:** Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Substantial exposure to any pesticide was positively associated with the risk of non-Hodgkin's lymphoma (odds ratio (OR) 3.09, 95% confidence interval (CI) 1.42, 6.70). Subjects with exposure to organochlorines, organophosphates, and ''other pesticides'' (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for ''other pesticides.'' None of the "higher" exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non-Hodgkin's lymphoma. **Conclusions:** The authors concluded that their findings of positive associations between pesticides and NHL were consistent with previous studies.

Materials and Methods

Subject selection: Cases were persons with incident non-Hodgkin's lymphomas that were diagnosed between January 1, 2000, and August 31, 2001, and reported to the Central Cancer Registry of New South Wales, Australia. Patients were 20–74 years of age and resident in New South Wales or the Australian Capital Territory. Ineligibility criteria included a history of organ transplantation or human immunodeficiency virus infection, poor English language skills, inability to complete a telephone interview, or a diagnosis of chronic lymphocytic leukemia, plasma cell myeloma, or B- or T-cell lymphoblastic leukemia. An anatomic pathologist reviewed all relevant pathology reports for all consenting patients. The pathologist reviewed diagnostic histopathology for all consenting patients judged to be less than 90% certain to have an eligible diagnosis of NHL in the report review. 1,217 NHL cases were originally deemed eligible for the study; 842 were eventually approached for an interview (69%), and 704 of those 842 (84%) were included in the analysis (overall, 58% of those initially thought to be eligible) (per Hughes et al. 2004).

Controls were randomly selected from the New South Wales and Australian Capital Territory electoral rolls to approximately match the expected distributions of cases with regard to age, sex, and region of residence (New South Wales or Australian Capital Territory). Similar eligibility criteria were used as for cases, except for human immunodeficiency virus infection, which was expected to be rare in the general population. 1,687 controls were originally deemed eligible for the study; 1,136 were eventually approached for an interview (67%), and 694 of 1,136 (61%) were included in the analysis (41% of those initially thought to be eligible) (per Hughes et al. 2004).

Exposure assessment: Cases and controls were interviewed with respect to their occupational histories and an industrial hygienist assigned pesticide exposures based on professional judgment and a crop-pesticide matrix.

Statistical analyses: Logistic regression was used to calculate ORs and 95% CIs. Final models controlled for age, sex, region, and ethnicity.

Results

Substantial exposure[4] to any pesticide was positively associated with the risk of non-Hodgkin's lymphoma (odds ratio (OR) 3.09, 95% confidence interval (CI) 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and ''other pesticides'' (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for ''other pesticides.'' None of the "higher" exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non-Hodgkin's lymphoma.

Discussion

The authors concluded that the increases in risk they observed are consistent with previous studies.

References

Hughes AM, Armstrong BK, Vajdic CM, et al. Pigmentary characteristics, sun sensitivity, and non-Hodgkin's lymphoma. Int J Cancer 2004; 110: 429-434.

4. Assessment and conclusion

Assessment and conclusion by applicant:

Fritschi et al. (2005) conducted an NHL case-control study to assess a possible relationship with pesticide exposures in Australia. The exposure assessment was indirect. No subject actually specified that they worked with glyphosate or any other individual pesticide. Exposures to pesticides and pesticide classes were assigned by an industrial hygienist based on judgment and a crop-pesticide matrix. The accuracy of this method of exposure assessment is unclear.

Substantial use of any pesticide was associated with NHL (OR = 3.1, 95% 1.4, 6.7). There were no analyses for glyphosate per se. The category that included glyphosate (and carbamates and other pesticides), "other pesticides", was associated with NHL – just as all the other pesticide sub-categories were. Analyses that considered likelihood and amount of pesticide exposure did not find associations with NHL.

This study has a number of major limitations. Foremost is the lack of specificity of the exposure assessment and the fact that there were no analyses of glyphosate per se or any other individual pesticide. Accordingly, it is impossible to judge any result as being attributable to glyphosate exposure or exposure to any other specific pesticide. Further, it hinders the interpretation of the positive associations with pesticides that they did not persist with higher likelihood or extent of exposure.

Second, participation by cases (84% of those approached) greatly exceeded that by controls (61% of those approached). Marked differences in participation between cases and controls raise concerns about selection bias: specifically, whether the exposure prevalence for participating controls is a valid estimate of the exposure prevalence in the population that gave rise to the cases (per Rothman KJ, Greenland S, Lash TL. Eds. 2008).

Another concern, as in all case-control studies of pesticides, is recall bias. The authors argued that their indirect method of exposure assessment, where subjects were not asked directly to name pesticides, would minimize recall bias. That is highly speculative. The fact that all categories of pesticides were associated with NHL would fit the paradigm for a study with recall bias.
Lastly, the statistical analysis only controlled for age, sex, region and ethnicity and did not control for other pesticide exposures. That is the classic scenario for uncontrolled confounding.

[4] Exposure was classified as substantial if the subject was probably exposed to the substance at a medium or high level for more than five 8-hour days per year for a combined total of more than 5 years

In conclusion, this study is not informative with respect to whether there is a relationship between glyphosate and NHL in the New South Wales population. The exposure assessment is speculative, the analysis does not specify glyphosate or any other particular pesticide, the analysis did not control for confounding by correlated exposures, and there are unresolved issues of selection bias and recall bias.

References

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Fritschi L, et al. Occupational Exposure to Pesticides and Risk of Non-Hodgkin's Lymphoma. Amer J Epidemiol 2005; 162: 849-857.	**Criteria met? Y/N/?**	**Comments** There were no analyses specific to glyphosate in this study.
Study Design		
Adequate study design given study objectives	No	There was no specificity with respect to glyphosate or other individual pesticides.
Appropriate study population to address potential glyphosate-related health outcomes	Yes	
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	
Comparable participation by groups being compared	No	Cases (84%) controls (61%)
Information provided by proxy respondents	No	
Adequate statistical analysis	No	No analyses for glyphosate per se.
Adequate consideration of personal confounding factors	No	
Adequate consideration of potentially confounding exposures	No	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	No analyses specific to glyphosate. Issues of recall bias, selection bias, and confounding.

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability low: case-control study, not adequately covering exposure assessment

Population : Reliability high : cases selected from Central Cancer Registry, controls from electoral rolls matching distribution of age, sex and region of residence.
Exposure assessment: Reliability low, questionnaire in which no question of specific pesticide exposure. Industrial hygienist assessed likelihood of exposure to substance based on occupational history. No assessment on an association with glyphosate can be made on the basis of this study.
Outcome assessment: Reliability high, cases selected from Central Cancer Registry and additional review of pathology report by pathologist to assure the correct diagnosis.
Confounder control: Reliability moderate: adjustments made for age sex, ethnic origin and state of residence. No adjustments made for potential confounders such as smoking, previous medical history.
Statistical methods : Reliability moderate, methods appropriate to the study design. However, no assessment made for glyphosate exposure, only combined exposure addressed.
Reporting: Reliability high, key elements of the material and methods and results are described.

Since only combined exposure is addressed the study is not able to show an association between glyphosate and NHL.

B.6.5.18.20. Supporting publications – Hardell, 1999

Data point	K-CA 5.5-046
Report author	Hardell L, Eriksson M.
Report year	1999
Report title	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides
Reference	Cancer 1999; 85:1353–1360
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: The cases and controls from this paper were included in the analysis by Hardell et al. (2002). The limitations reported there also apply to this study (Hardell and Eriksson, 1999). It is noted that for glyphosate there were only 4 exposed cases and 3 exposed controls limiting the reliability of the study.

2. Full summary of the study according to OECD format

The cases and controls from this study were included in a pooled analysis by Hardell et al. (2002). Please refer to the review of that paper.

5. Assessment and conclusion

Assessment and conclusion by applicant:

Please see assessment of Hardell et al. 2002.

Reference
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; 43:1043-1049.

Reliability Criteria: Epidemiology studies

Publication: Hardell L, Eriksson M. A case-control study of non-Hodgkin's lymphoma and exposure to pesticides. Cancer 1999; 85: 1353-1360.	**Criteria met? Y/N/?**	**Comments** The cases and controls from this study were included in a pooled analysis by Hardell et al. (2002). Please refer to the review of that paper.
Study Design		
Adequate study design given study objectives		
Appropriate study population to address potential glyphosate-related health outcomes		
Exposure studied		
Exposure to formulations with glyphosate as a.i.		
Exposure to formulations with other a.i.		
Exposure to other farm exposures		
Study Conduct/analysis		
Adequate description of study population		
Adequate description of exposure circumstances		
Comparable participation by groups being compared		
Information provided by proxy respondents		
Adequate statistical analysis		
Adequate consideration of personal confounding factors		
Adequate consideration of potentially confounding exposures		
Overall assessment		
Reliable without restrictions		
Reliable with restrictions		
Not reliable		

Assessment and conclusion by RMS:

The cases and controls from this paper were included in the analysis by Hardell et al. (2002) which is evaluated in section B.6.5.18.21. It is however noted that for glyphosate there were only 4 exposed cases and 3 exposed controls limiting the reliability of the study.

B.6.5.18.21. Supporting publications – Hardell, 2002

Data point	KCA 5.5-045
Report author	Hardell L, Eriksson M, Nordstrom M.
Report year	2002
Report title	Exposure to pesticides as a risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies
Reference	Leukemia and Lymphoma 2002; 43(5): 1043-1049
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, RAR 2015 (study concluded to be not reliable)
GLP/Officially recognized testing facilities	Not applicable

Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: The study is concluded to be of low reliability (see assessment RMS at the end of the study summary).

Full summary of the study according to OECD format

The authors conducted a pooled analysis of two Swedish case-control studies to study the relationship between exposure to phenoxyacetic acids and other pesticides for non-Hodgkin's lymphoma (NHL) (Hardell and Eriksson 1999) and hairy cell leukemia (HCL), a rare subtype of NHL (Nordstrom et al. 1998). **Methods:** The studies that were pooled were population based with cases identified from a cancer registry and controls identified from a population registry. Data assessment involved a mailed questionnaire supplemented by telephone interviews. **Statistical analysis:** Conditional logistic regression was implemented to estimate odds ratios (ORs) and 95% confidence intervals (CIs). ORs for specific pesticides employed subjects with no pesticide exposures as the referent. **Results:** The pooled analysis included 515 cases and 1,141 controls. Elevated ORs in univariate analyses for specific pesticides were found for glyphosate (OR = 3.04, 95% CI 1.08, 8.52), and MCPA (OR = 2.62, 95% CI 1.40, 4.88). For several categories of pesticides, the biggest risk was found for exposure during the latest decades before diagnosis. However, in multivariate analysis, the only significantly increased risk was for a heterogeneous category of herbicides other than those specified above. ORs for these exposures in multivariate analyses were: glyphosate (OR = 1.85, 95% CI 0.55, 6.20), MCPA (OR = 1.67, 95% CI 0.77, 3.57).

Materials and Methods

Subject selection: NHL cases were identified from the national cancer registry during the period 1987 – 1990 in the four most northern counties of Sweden and three counties in mid-Sweden. All cases were histologically verified. Of the total 442 NHL cases, 192 were deceased. HCL cases (n = 121) were identified from the national cancer registry during the period 1987-1992 and comprised those in the entire country. Of the 442 NHL cases, 404 (91%) completed the study questionnaire and were included in the study. Of the 121 HCL cases, 111 (91%) were included in the study. Deceased cases were excluded. In total, therefore, there were 515 cases included in the pooled analysis.

For living NHL cases, two male controls matched for age, and county were recruited from the national population registry. For each deceased NHL case, two deceased controls matched for age, county, and year of death were identified from the national death registry. For deceased subjects, information was gathered from next-of-kin. For HCL cases, four controls matched for age and county were drawn from the national population registry. Participation among potential controls was 83%, yielding a control population of 1,141.

Exposure assessment: Study subjects or next-of-kin received a mailed questionnaire that requested a complete occupational history for the study subject as well as exposure to different chemicals. Years and total exposure days were requested for specific chemicals. If the responses were unclear, an interviewer contacted the respondent over the phone for clarifications. It is unclear how frequently responses to mailed questionnaires had to be clarified via telephone interviews.

Statistical analyses:

Univariate and multivariate conditional logistic regression was implemented to estimate odds ratios (ORs) and 95% confidence intervals (CIs). The authors did not describe how variables for the multivariate analyses were determined. ORs for specific pesticides were calculated with subjects with no pesticide exposures as the referent.

Results

A number of pesticides and pesticide classes showed significantly elevated ORs in univariate analyses. However, ORs were reduced appreciably in multivariate analyses and only the "other herbicides" category has a significantly elevated OR of 2.3 (95% CI 1.0, 5.2). For glyphosate, the univariate OR was 3.0 (95% CI 1.1, 8.5) and the multivariate OR was 1.9 (95% CI 0.6, 6.2).

Discussion

The authors concluded that their analyses shed further light on the etiology of NHL. They speculated that immunomodulation by pesticides might be an underlying mechanism, perhaps in combination with latent Epstein-Barr virus infection. They also speculated that a short induction period might be operative with pesticides (not specified as to which ones).

References

Hardell, L.; Eriksson, M. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer. 1999, 85, 1353–1360.

Nordstrom, 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, 77, 2048–2052.

Assessment and conclusion

Assessment and conclusion by applicant:

The pooled analysis by Hardell and Eriksson is one of several case-control studies in the glyphosate literature. The adjusted OR for glyphosate in this pooled analysis was 1.9 (95% CI 0.6, 6.2) and, taken at face value, would not support the conclusion of relationship between glyphosate and NHL/HCL.

This study has many of the same limitations as other case-control studies in the glyphosate literature. Foremost, is the very large amount of information on pesticide use that was obtained from proxy respondents – 44% for cases and, though not specified by the authors, it has been assumed by Chang and Delzell (2016) to be 44% for controls based on the vital status matching employed for NHL cases. It is an important limitation of this study that results were not presented stratified by type of respondent. There are many examples in the pesticide epidemiologic literature where ORs based on proxy respondents differ markedly from those based on self-respondents (Lee et al. 2005; Johnson et al. 1993.)

Potential proxy response bias is a part of the larger issue of recall bias in this study. Crump conducted an analysis to try to discern whether recall bias and/or selection bias were operative in the case-control studies that are part of the glyphosate epidemiologic literature. His thesis was that since the studies that included glyphosate also included many other pesticides, one could get a measure of whether the methodology was biased by assessing the proportions of non-glyphosate ORs that were greater than the null value of 1.0. If the methodology was unbiased, the proportion of ORs greater than 1.0 for non-glyphosate exposures should be approximately 50%. For Hardell and Eriksson (2002), the percent of non-glyphosate ORs that were greater than 1.0 was 90%. As a result, Crump (2020) concluded that studies like Hardell and Eriksson (2002) with such a marked skew toward positive results for non-glyphosate exposures are not reliable for determining validly exposure-disease relationships for glyphosate.

There are also a number of questions about how the univariate and multivariate analyses were conducted. In both univariate and multivariate analyses, in order to be in the unexposed referent group, one could not have reported exposure to any pesticide. So, for example, in the analysis for glyphosate, all of the individuals who had reported exposure to pesticides other than glyphosate were excluded from the analysis. According to Table 1 in Hardell and Eriksson (2002), a substantial number of individuals would have been excluded from the glyphosate analysis due to exposure to other pesticides. According to accepted case control theory (Rothman et al. 2008), the validity of case control studies depends on accurately estimating the exposure prevalence in the population that gave rise to the cases. Clearly, those who used pesticides other than glyphosate are part of the population that gave rise to the cases in this study. Exposure prevalence cannot be estimated accurately by excluding from the reference group those with farm exposures other than the exposure of interest. This practice distorts exposure prevalences and can bias OR estimates (see a numerical illustration in Acquavella et al. 2016). The exclusions also preclude being able to adequately control for the potential confounding of other pesticide exposures (Chang and Delzell 2016). Another analysis concern is the lack of detail about how confounders in the multivariate models were determined. More specification should have been provided.

In conclusion, this study has an equivocal result for glyphosate and NHL. That result is likely not interpretable at face value due to strong indications of recall bias and issues relating to the conduct of the analysis.

References

Acquavella J, Garabrant D, Marsh G, et al. Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma. Critical Rev Toxicol 2016:46(S1):28-43.
Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.
Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Anal 2020; 40(4): 696-704.
Johnson RA, Mandel JS, Gibson RW, et al. Data on Prior Pesticide Use Collected from Self-and Proxy Respondents. Epidemiology 1993; 4:157-164.

Lee WJ, Colt JS, Heineman EF, et al. Agricultural pesticide use and risk of glioma in Nebraska, United States. Occup Environ Med. 2005; 62:786–792.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Reliability Criteria: Epidemiology studies

Publication: Hardell L, Eriksson M, Nordstrom M. Exposure to pesticides as a risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies. Leukemia and Lymphoma 2002: 43(5): 1043-1049.	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	No	Recall bias, residual confounding, high proportion of proxy respondents.
Appropriate study population to address potential glyphosate-related health outcomes	Yes	
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	Though not addressed in any analyses
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	Yes	
Adequate statistical analysis	No	Uncertain assessment of confounding for glyphosate. Analytic selection bias.
Adequate consideration of personal confounding factors	Uncertain	Lack of specification about personal factors in the paper.
Adequate consideration of potentially confounding exposures	No	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability moderate, case control study, potential for recall bias.
Population: Reliability high, adequate inclusion method of cases and controls.
Exposure assessment: Reliability moderate to low, questionnaire answered by subjects or proxy. Potential for recall bias which is evidenced by the high odds ratios for virtually all pesticides evaluated. Fairly high percentage of questionnaires were answered by proxy.
Outcome assessment: Reliability moderate, cases were verified by histopathology. However, number of glyphosate exposed cases and controls are somewhat low (8 each).
Confounder control: Reliability low, no adjustment for life style factors, such as smoking, or previous medical history appears to have carried out.
Statistical methods Reliability low, in the univariate analysis for the individual pesticides the unexposed category consisted of subjects that were unexposed to all included pesticides which can be expected to lead to

differences between the groups based on other covariates. It would have been more accurate to compare to groups that use other pesticides. Furthermore the analytical methods are not described in detail.
Reporting: Reliability low, description of statistical analysis was very limited. It is unclear which adjustments were made for confounders.

Overall conclusion : Based on the limitations discussed above the study is concluded to be of low reliability.

B.6.5.18.22. Supporting publications – Lee, 2005

Data point	KCA 5.5-047
Report author	Lee et al.
Report year	2005
Report title	Agricultural pesticide use and risk of glioma in Nebraska, United States
Reference	Occup Environ Med 2005; 62:786-792
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, RAR 2015
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Study reliable, although the study was a case-control study which have to potential for recall bias it is well conducted and described in the publication.

Full summary of the study according to OECD format

Background: The authors sought to evaluate the risk of the adult glioma associated with farming and agricultural pesticide use in a population-based case control study in eastern Nebraska. **Methods:** Telephone interviews were conducted with men and women diagnosed with gliomas (n = 251) between 1988 and 1993 and controls (n = 498) randomly selected from the same geographical area. **Statistical analysis:** Unconditional logistic regression was used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for farming and for use of specific pesticides and pesticide classes. **Results:** Among men, working on a farm was with a significantly increased risk of glioma (>55 years on a farm OR= 3.9, 95% CI 1.8 to 8.6); however, positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers; however, most of the positive associations were limited to proxy respondents. For two herbicides and three insecticides, use was positively associated with risk among both self and proxy respondents: for the herbicides metribuzin (OR = 3.4, 95% CI 1.2 to 9.7) and paraquat (OR = 11.1, 95% CI 1.2 to 101), and for the insecticides bufencarb (OR = 18.9, 95% CI 1.9 to 187), chlorpyrifos (OR = 22.6, 95% CI 2.7 to 191), and coumaphos (OR = 5.9, 95% CI 1.1 to 32). For males, the OR for glyphosate was 1.5 (OR = 1.5, 95% CI 0.7, 3.1) resulting from an OR of 3.1 (95% CI 1.2, 8.2) based on proxy responses and an OR of 0.4 (95% CI 0.1, 1.6) based on self-respondents. **Conclusion:** The authors concluded while they found some positive associations for specific pesticide exposures, most were limited to data provided by proxy respondents. They considered that their findings warrant further evaluation in prospective cohort studies where issues of recall bias are not a concern.

Background

The authors conducted a population-based case-control study in Nebraska to determine if agricultural pesticide exposures were associated with the risk of adult glioma. They had a specific interest in evaluating risk from pesticides believed to be nitrosatable (able to form N-nitroso compounds upon reaction with nitrite).

Materials and Methods

Subject selection: Participants were white male or female residents of 66 Nebraska counties, aged 21 years or older. Cases were histologically confirmed incident primary adult gliomas diagnosed between 1 July 1988 and 30 June 1993 and were identified from the Nebraska Cancer Registry, or from 11 participating hospitals in Lincoln and Omaha covering more than 94% of adult glioma cases in the study population.

Controls were randomly selected from the control group of a previous population-based case control study of non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, and chronic lymphocytic leukemia conducted in 1986–87 in the same base population (Zahm et al. 1990) matched by age, sex, and vital status. Participation for cases was 89% and participation for controls was 83%.

Exposure ascertainment: Glioma cases, controls, or their proxies were interviewed by telephone during 1992–94 using a structured questionnaire containing information about demographics, smoking and alcohol consumption, diet, family history of cancer, complete residential and occupational history, medical history, and other factors. Among those who lived or worked on a farm, the authors obtained a detailed history of pesticide use on the farm as well as years of farming activity. Subjects were queried about the use of specific pesticides based on a list developed by local experts.

Given the short survival for glioma patients, case interviews were completed primarily by proxies (76%). Most proxy respondents were spouses (62%) or other first-degree relatives (33%). Among controls, 60% of the interviews were conducted with proxies. Proxy respondents were primarily spouses (45%) or other first-degree relatives (46%).

Statistical analyses: The authors used unconditional logistic regression to calculate ORs and 95% CIs. The ORs for farming activity and pesticide use were calculated using non-farmers as a reference group. Subjects who lived or worked on a farm only before age 18 (n=145) were evaluated separately because there farming experience and pesticide use was generally low. Odds ratios were adjusted for age ((49, 50–59, 60–69,>70), sex, and respondent type. Confounders variables evaluated that were considered to be potentially associated with glioma were a history of head injury, marital status, education level, alcohol consumption, medical history of diabetes mellitus, dietary intake of alpha- and beta-carotene and dietary fibre. None of these factors changed the OR by more than 10% and the final models were adjusted only for age, sex and respondent type. Trend tests were performed by assigning scores to categorical variables using the median value among controls and treating the scored variables as continuous in the logistic analyses.

Results

Overall, brain cancer risk was increased among farmers, however, the increased risk was only observed based on proxy responses. There were no increased risks of glioma among women who lived or worked on a farm and the ORs were similar for proxy and self-respondents.

Brain cancer risk was increased among adult male farmers reporting that insecticides (OR=1.8, CI 1.0 to 3.0), herbicides (OR=1.7, CI 1.0 to 3.0), or nitrosatable pesticides (OR=1.9, CI 1.1 to 3.4) were used on the farm on which they lived or worked. The increased risks were limited to proxy respondents. Among women, there were no significant associations for any of the pesticide classes, regardless of respondent type.

The OR for glyphosate was 1.5 (OR = 1.5, 95% CI 0.7, 3.1) resulting from an OR of 0.4 (95% CI 0.1, 1.6) based on self-respondents an OR of 3.1 (95% CI 1.2, 8.2) based on proxy responses.

Discussion

The authors concluded that they found positive associations between agricultural pesticide use and the risk of brain cancer, primarily based on proxy responses, leading to the concern that many of the associations found were due to misclassification of exposure by proxy respondents. The authors concluded that the positive associations observed warrant further evaluation, particularly in prospective cohort studies where issues of recall bias are not a concern.

References

Zahm SH, Weisenburger DD, Babbitt PA, et al. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4- dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1990; 1:349–356.

Assessment and conclusion by applicant:

The case-control study by Lee et al. (2005) is most notable because it evaluated the impact of getting information from proxy versus self-respondents. It is remarkable how frequently the ORs based on proxy responses were markedly different, mostly higher, than ORs based on self-reported information. A previous evaluation by Johnson et al. (1993) also showed that pesticide data provided by proxy respondents can result in higher ORs compared with data provided by self-respondents, but the frequency of divergent ORs was not as high as that in Lee et al. In studies with a substantial proportion of proxy respondents, like several of the glyphosate case-control studies, results should be presented separately by type of respondent to assess the potential for proxy reporting bias.

The glyphosate results in this study were consistent with the overall pattern of markedly higher ORs based on proxy respondents. The OR based on self-respondents was 0.4 (95% CI 0.1, 1.6), while the OR based on proxy respondents was 3.1 (95% CI 1.2, 8.2).

Lee et al. (2005), like other case-control studies, needs to be evaluated for issues of selection bias, recall bias, and confounding. With respect to selection bias, case and control participation were pretty similar (89% and 83%, respectively). However, there were more proxy respondents for cases (76%) than controls (60%). Lee et al. (2005) were transparent on this account, so the divergence in results based on self and proxy respondents was amply illustrated. Nonetheless, there is still the possibility of recall bias among self-respondents. It's difficult to demonstrate such recall bias without access to a gold standard for comparison with the recalled exposures.

Confounding bias is also a potential concern in this study because ORs for specific pesticides were only adjusted for age, sex and respondent type and not for other pesticides. However, the normal concerns about confounding take a back seat to the aforementioned validity issues related to the high proportion of proxy respondents, especially for cases.

There is another type of bias of concern in this study, sometimes called analytic selection bias (see a numerical illustration in Acquavella et al. 2016; also noted in Chang and Delzell 2016). This type of bias occurs when non-farmers are used as the referent group for pesticide specific analyses. According to accepted case control theory (Rothman et al. 2008), the validity of case control studies depends on accurately estimating the exposure prevalence in the population that gave rise to the cases. Exposure prevalence cannot be estimated accurately by excluding from the reference group those with farm exposures other than the exposure of interest. This practice distorts exposure prevalences and can bias OR estimates. Again, the potential for this type of bias takes a back seat to the validity issues related to proxy respondents in this study.

In conclusion, this study does not provide evidence of a positive relationship between glyphosate exposure and glioma. Based on self-respondents, the OR for glyphosate was 0.4 (95% CI 0.1, 1.6). The authors are to be congratulated for a clear exposition of the differences in results based on proxy and self-respondents. That being said, the study has other unaddressed validity concerns that are typical for pesticide case-control studies.

References

Acquavella J, Garabrant D, Marsh G, et al. Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma. Critical Rev Toxicol 2016:46(S1):28-43.

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.

Johnson RA, Mandel JS, Gibson RW, et al. Data on Prior Pesticide Use Collected from Self-and Proxy Respondents. Epidemiology 1993; 4:157-164.

Lee WJ, Colt JS, Heineman EF, et al. Agricultural pesticide use and risk of glioma in Nebraska, United States. Occup Environ Med. 2005; 62:786–792.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Reliability Criteria: Epidemiology studies made by applicant

Publication: Lee WJ, Colt JS, Heineman EF, et al. Agricultural pesticide use and risk of glioma in Nebraska, United States. Occup Environ Med. 2005; 62:786–792.	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	No	High proportion of proxy respondents.
Appropriate study population to address potential glyphosate-related health outcomes	No	High fatality rate precluded collecting accurate exposure information.
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	High proportion of proxy respondents.
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	Yes	76% for cases, 60% for controls
Adequate statistical analysis	No	Helpful to provide results by type of respondent, but no control for confounding by other exposures and inappropriate referent group (those with no pesticide exposure).
Adequate consideration of personal confounding factors	No	
Adequate consideration of potentially confounding exposures	Uncertain	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	But helpful regarding issues with proxy respondents.

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability moderate, case control study
Population: Reliability high, study population well described. Study population covered more than 94% of adult glioma cases in the study population. Random sampling for controls.
Exposure assessment: Reliability moderate, questionnaire answered by subjects or proxy. High percentage of questionnaires were answered by proxy.
Outcome assessment: Reliability high, case inclusion based on Nebraska Cancer Registry or 11 participating hospitals.
Confounder control: Reliability high, the effect of possible confounders were evaluated including history of head injury, marital status, education level, alcohol consumption, medical history of diabetes mellitus, dietary intake of alpha- and beta-carotene and dietary fibre. Since none of these factors changed the ORs by more than 10% the final models were only adjusted for age, sex and respondent type.

Statistical methods : Reliability high, the statistical methods were appropriate to the study design. To address the high number of proxy respondents authors calculated overall as well as separate ORs for self-responders and proxy responders.
Reporting: Reliability high : Key elements of the material and methods and results section are adequately described.

Unlike the applicants, the RMS does not automatically consider all case-control studies to be unreliable due to the potential of recall bias. The study is well conducted and described. The study authors are clear about the limitations of their study and do not make unfounded conclusion on the basis of the results. The study did have a high number of proxy responders, but the study authors are clear about this issue and calculated separate ORs for both self-respondents and proxy responders. As the study authors also indicate the difference between the ORs of self-respondents (not stat. significant) and proxy respondent (stat. significant) is striking and may be due to misclassification by proxy respondents. The RMS agrees that this may come from either information bias (knowing status of the self-respondents) or/and exaggerated exposure response in proxy group or/and lower accurate reporting in self-respondent group in comparison to the proxy-group. However, another interpretation could be that reaching proxies may not result in an actual bias but simply their attention was caught and a more accurate reporting from proxy group occurred i.e., whilst self-respondent exposure was genuinely underestimated and proxy reply was correct. In theory, and since this study is reliable and since adjustments seem to have been adequately included (age, sex, etc…) study authors should have undertaken further investigation.
As also indicated by the study authors further research is needed to draw clear conclusions on the potential associations observed in the study.

B.6.5.18.23. Supporting publications – McDuffie, 2001

Data point	KCA 5.5-048
Report author	McDuffie et al.
Report year	2001
Report title	Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men: Cross-Canada Study of Pesticides and Health.
Reference	Cancer Epidemiol Biomark Prev 2001; 10:1155–1163
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognized testing facilities	Not applicable
Previous evaluation	Yes, RAR 2015
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: Reliable with restrictions (see assessment made by RMS at the end of the study summary).

Full summary of the study according to OECD format

McDuffie et al. (2001) conducted a trans-Canadian multi-center case control study to evaluate the relationship between pesticide exposures and non-Hodgkin's lymphoma (NHL). **Methods:** Cases (n = 517) were identified from provincial Cancer Registries except in Quebec, for which hospital ascertainment was used. Controls (n = 1506) were selected at random from the provincial Health Insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia). Participation was 67% for cases and 48% for controls. Pesticide exposure was determined through telephone interview of study participants or their proxies. **Statistical Analysis:** The authors used conditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CIs). **Results:** The adjusted OR for any reported glyphosate use was 1.2

(95% CI 0.8 to 1.7) controlling for age, province, and significant medical variables, such as family history of cancer. The strongest pesticide associations were with mecoprop (OR = 2.3) and dicamba (OR = 1.9). A subsequent analysis for individual pesticides by days of use (none, ≤ 2 days/year, > 2 days/year) showed glyphosate ORs of 1.0, 1.0 (0.6 to 1.6), and 2.1 (95% CI 1.3 to 2.7). This latter analysis did not adjust for the medical variables as in the dichotomous exposure analysis or for the potential confounding effects of other pesticides. **Conclusions:** The authors concluded that NHL was associated with specific pesticides after adjustment for other independent predictors.

Materials and Methods

Subjects and ethics statement: Cases were ascertained from provincial Cancer Registries except in Quebec, for which hospital ascertainment was used. The Cancer Registries and hospitals provided information, including pathology reports, to confirm diagnosis. Controls were selected at random from the provincial Health Insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia). Pesticide exposure was determined by via questionnaire-based interviews. The questionnaires were modified versions of the National Cancer Institute (NCI) telephone interview questionnaire that were used in the NCI Kansas and Nebraska case control studies (Hoar et al. 1986; Zahm et al. 1990). Each subject who reported 10 h per year or more of exposure to pesticides (any combination of compounds) as defined by the screening questions, and a 15% random sample of the remainder was mailed a list of pesticides and interviewed to get details of pesticide use. There were apparently some proxy respondents, but the authors did not specify the number and whether proxy respondents were more common among cases than controls.[5] The authors did mention that they minimized proxy respondents by excluding decedents from the potential case and control populations.

Statistical analyses: In dichotomous (viz., ever versus never) exposure analyses, the authors used conditional logistic regression to estimate ORs and 95% CIs controlling for age, province, and statistically significant medical variables related to NHL (i.e. history of measles, mumps, cancer, allergy desensitization shots, and a positive family history of cancer in a first-degree relative). Stratified analyses were also presented for individual chemicals by days of use categories with control for age and province of residence. These analyses did not control for significant medical variables related to NHL or for the effects of other pesticides. Statistical tests for trend were not conducted.

Results

The OR for any reported glyphosate use was 1.2 (95% CI 0.8 to 1.7) controlling for age, province, and significant medical variables. The strongest pesticide associations were with mecoprop (OR = 2.3) and dicamba (OR = 1.9); both pesticides were used more frequently by cases than glyphosate (14.1% and 10.2% versus 9.9%). The dichotomous glyphosate result was not adjusted for these or other pesticides. A subsequent analysis for individual pesticides by days of use (none, ≤ 2 days/year, > 2 days/year) showed glyphosate ORs of 1.0, 1.0 (0.6 to 1.6), and 2.1 (95% CI 1.2 to 3.73). In their final statistical models, NHL was associated with a personal history of cancer, a history of cancer in first-degree relatives, and exposure to dicamba-containing herbicides, to mecoprop, and to aldrin. A personal history of measles and of allergy desensitization treatments was associated with a lowered risk.

Discussion

The authors concluded that their results support previous findings of an association between NHL and specific pesticide exposures.

References

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.

[5] Chang and Delzell characterized 21% of the case respondents as proxies versus 15% of the control respondents (Chang and Delzell 2016).

Hoar SK, Blair A, Holmes F, et al. Agricultural herbicide use and risk of lymphoma and soft tissue sarcoma. J. Am. Med. Assn., 256: 1141–1147, 1986.

Zahm SH, Weisenburger DD, Babbit PA, et al. A case control study of non-Hodgkin's lymphoma and agricultural factors in Eastern Nebraska. Epidemiology, 1: 349–356, 1990.

Assessment and conclusion by applicant:

McDuffie et al. (2001) conducted a large, trans-Canada case-control NHL study (n = 517 cases) to evaluate associations between pesticides and NHL. Focusing on the results for glyphosate, the OR for any reported glyphosate use was 1.2 (95% CI 0.8 to 1.7) controlling for age, province, and significant medical variables. In similar dichotomous analyses, stronger associations were seen for mecoprop (OR = 2.3, 95% CI 1.6, 3.4), dicamba (OR = 1.9, 95% CI 1.3, 2.7)) and phenoxy herbicides (OR = 1.4, 95% CI 1.1, 1.8) and these pesticides that were used more frequently by cases than glyphosate (14.1%, 10.2%, and 25.3%, respectively, versus 9.9% for glyphosate). The dichotomous glyphosate result was not adjusted for these or other pesticides. A subsequent analysis for individual pesticides by days of use (categories were none, ≤ 2 days/year, > 2 days/year) showed glyphosate ORs of 1.0, 1.0 (95% CI 0.6 to 1.6), and 2.1 (95% CI 1.3 to 2.7). These ORs were adjusted only for age and province. The authors called this a dose response relationship, but statistical tests for trend were not conducted and, notably, the glyphosate ORs were not adjusted for other pesticides or for the medical variables that were controlled in the dichotomous ever-exposed analysis. In the authors' final statistical models, NHL was associated with a personal history of cancer, a history of cancer in first-degree relatives, and exposure to dicamba-containing herbicides, to mecoprop, and to aldrin. A personal history of measles and of allergy desensitization treatments was associated with a lowered risk. Glyphosate was not reported as a risk factor in the final statistical models.

McDuffie et al. (2001) employed a case control design for their study. Epidemiology textbooks describe the case control design as related to the cohort study design – the fundamental design for epidemiologic research. In fact, a case control study is best thought of as a cohort study for a source population (viz., the conceptual cohort) where the cases are detected during a specified time period and the controls are a sample of the concurrent source population that gave rise to the cases (Rothman KJ, Greenland S, Lash T eds. 2008). Case control studies can give valid exposure-disease estimates if, by virtue of study design or through analytic methods, they obviate issues of selection bias, recall bias, and confounding.

With respect to selection bias, a fundamental principle in case control studies is that controls need to be an unbiased sample of the source population that gave rise to the cases. That is necessary for the controls to provide a valid estimate of the source population's exposure prevalence so that the exposure odds ratio (OR) is a valid estimate of the ratio of disease rates (or relative risk (RR)) for exposed versus unexposed individuals. In case control studies, lesser participation by controls can result in an inaccurate estimate of the exposure prevalence for the source population and result in a biased OR. Differential participation was substantial in this study with much lower participation for controls (48%) than cases (67%).

Recall bias has been noted repeatedly as an important, often intractable concern in case control studies of pesticides (Chang and Delzell 2016; Acquavella et al. 2016). In fact, the United States Agricultural Health Study – a prospective cohort study – was launched due to the perceived need to obviate recall bias with a prospective orientation between exposure assessment and disease identification (Alavanja et al. 1994). It's difficult to demonstrate recall bias without access to a gold standard for comparison with the recalled exposure.

Crump (2020) conducted an analysis to try to discern whether recall bias and/or selection bias were operative in the case-control studies that are part of the glyphosate epidemiologic literature. His thesis was that since the studies that evaluated glyphosate also evaluated many other pesticides, one could get a measure of whether the methodology was biased by assessing the proportions of non-glyphosate ORs that were greater than the null value of 1.0. If the methodology was unbiased, the proportion of ORs greater than 1.0 for non-glyphosate exposures should be approximately 50%. For McDuffie et al. (2001), the percent of non-glyphosate ORs that were greater than 1.0 was 93%. As a result, Crump (2020) concluded that studies like McDuffie et al. with such a marked skew toward positive results for non-glyphosate exposures are not reliable for determining validly exposure-disease relationships for glyphosate.

Recently Pahwa et al. (2019) conducted a pooled reanalysis of the NHL data from McDuffie et al. (2001) and DeRoos et al. (2003) case control studies. The reanalysis sought to evaluate associations for glyphosate use and NHL implementing more extensive control of confounding factors than in the original publications and considering the impact of excluding pesticide information provided by next-of-kin or proxy respondents. The pooled OR for NHL overall for any use of glyphosate was 1.4 (95% CI 1.1, 1.8). After adjustment for other pesticides, the OR was reduced to 1.1 (95% CI 0.8, 1.5) and the OR was 0.95 (95% CI 0.7, 1.3) after excluding data from proxy respondents [supplemental table 1 in the article]. In analyses that considered the reported amount of exposure, ORs for glyphosate and NHL were not elevated in the higher categories for years of use (> 3.5 years OR 0.9, 95% CI 0.6, 1.4) or lifetime days of use (> 7 days OR 1.1, 95% CI 0.7, 1.8), though there was an elevated OR for the category of more than 2 days of use/year (OR 1.7, 95% CI 1.0, 2.9). The major limitations of the pooled reanalysis are the inability to address recall bias and selection bias in the original studies.

In conclusion, the study by McDuffie et al. (2001) appears to have major limitations with respect to selection bias and recall bias and the analyses for glyphosate did not address confounding by other pesticides. Subsequent publications by Crump (2020) and Parwa et al. support the view that the McDuffie et al. (2001) results are not reliable for assessing the putative relationship between glyphosate use and NHL.

References

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Pahwa M, Freeman LB, Spinelli JJ, et al. Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project. Scand J Work Environ Hlth. 2019;45(6):600–609.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Solomon K. Glyphosate in the general population and in applicators: a critical review of studies on exposures. Crit Rev Toxicol 2016; 46 suppl 1:21-27.

Reliability Criteria: Epidemiology studies made by applicant

Publication: McDuffie HH, Pahwa P, McLaughlin JR, et al. Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men: Cross-Canada Study of Pesticides and Health. Cancer Epidemiol Biomark Prev 2001; 10:1155–1163	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	No	Selection bias, recall bias, residual confounding, proxy respondents.
Appropriate study population to address potential glyphosate-related health outcomes	Yes	
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	Though not addressed in any analyses
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	
Comparable participation by groups being compared	No	
Information provided by proxy respondents	Yes	
Adequate statistical analysis	No	Limited assessment of confounding for glyphosate.
Adequate consideration of personal confounding factors	Yes	But, not uniformly applied across the various analyses
Adequate consideration of potentially confounding exposures	No	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability moderate: case control study.
Population: Reliability moderate: random sampling, high number of glyphosate exposed cases and controls. However, much lower response rate in controls than in cases.
Exposure assessment: Reliability moderate: questionnaire was evaluated in a validation pilot study by checking the response in 27 volunteer farmers with their records of purchases through their local agrochemical supplier. The questionnaire was not validated for other groups, e.g. amateur pesticide users.
Outcome assessment: Reliability high: cases from Cancer Registries, pathology report provided by Cancer Registries and hospitals were assessed by a pathologist.
Confounder control: Reliability moderate to low: ORs were adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization shots and positive family history of concern in a first-degree relative). However, the ORs that were calculated for number of days exposed per year which were the only ORs that were found statistically significant and were only adjusted for the variables age and province of residence.
Statistical methods: Reliability moderate: adequate statistical methods used for the overall glyphosate exposure. No adjustment made for confounders for the ORs differentiated based on frequency of exposure.
Reporting: Reliability high: Key elements of the materials and methods and results section are sufficient in detail.

Overall, the RMS concluded the reliability of the study to be moderate (reliable with restrictions). The main limitations to the study noted are:
1) the lack of adjustment for confounding in the ORs that were stratified by the number of days per year of exposure.
2) As the authors also noted a case-control design introduces the potential for recall bias and misclassification. While a pilot validation study was conducted in volunteer farmers other occupations and home and garden users were not part of the validation.
3) The low response rate, in particular in controls.

The adjusted OR for any reported glyphosate use was not statistically elevated with an OR of 1.2 (95%-CI 0.8-1.7). Analysis for glyphosate use by days of use did show a significantly elevated OR for >2 days of exposure/year with an OR of 2.1 (95%-CI 1.20-3.73). However, it is noted that no adjustments for confounders were made in this analysis (except for age and province of residence).

B.6.5.18.24. Supporting publications – Monge, 2007

Data point	KCA 5.5-049
Report author	Monge P, et al.
Report year	2007
Report title	Parental occupational exposure to pesticides and risk of childhood leukemia in Costa Rica
Reference	Scand J Work Environ Health 2007; 33(4): 293-303
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, RAR 2015
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No

	Conclusion AGG: Study concluded to be of low reliability (see assessment RMS at the end of the study summary).

Full summary of the study according to OECD format The authors studied parental exposure to pesticides and the risk of leukemia in offspring. The study setting was Costa Rica and the study design was a population-based case–control study. **Methods:** Cases of childhood leukemia (N=334) in 1995–2000 were identified at the Cancer Registry and the Children's Hospital. Population controls (N=579) were drawn from the National Birth Registry. Interviews of parents were conducted using conventional and icon-based calendar forms. An exposure model was constructed for 25 pesticides in five time periods. **Statistical analysis:** Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for exposures during 5-time windows. **Results**: Mothers' exposures to any pesticides during the year before conception (odds ratio (OR) 2.4, 95% confidence interval (95% CI) 1.0–5.9) and during the first (OR 22, 95% CI 2.8–171.5) and second (OR 4.5, 95% CI 1.4–14.7) trimesters were positively associated with increased leukemia risk. An association was found for fathers' exposures to any pesticides during the second trimester (OR 1.5, 95% CI 1.0–2.3). An increased risk with respect to organophosphates was found for mothers during the first trimester (OR 3.5, 95% CI 1.0–12.2) and for fathers during the year before conception and the first trimester (OR 1.5, 95% CI 1.0–2.2 and OR 1.6, 95% CI 1.0–2.6, respectively), and benzimidazoles during the first, second, and third trimesters of pregnancy (OR 2.2, 95% CI 1.0–4.4; OR 2.2, 95% CI 1.0–5.0; OR 2.2, 95% CI 1.0–5.2, respectively). There was a suggestion of an exposure–response gradient for fathers as regards picloram, benomyl, and paraquat. Age at diagnosis was positively associated with fathers' exposures and inversely associated with mothers' exposures. **Conclusions** The results suggest that parental exposure to certain pesticides may increase the risk of leukemia in offspring.

Materials and Methods

Subjects selection: All cases of childhood leukemia (ages 0–14 years at diagnosis, N=334) diagnosed in Costa Rica in 1995–2000 were identified at the Cancer Registry and the Children's Hospital of Costa Rica. Population controls (N=579), frequency matched to the cases by birth year, were drawn from the National Birth Registry. The overall response rate was 90% for the cases and 90.5% for the controls.

Exposure assessment: The authors conducted face-to-face interviews with parents in 2001–2003 to collect demographic data and data on known and suspected risk factors for childhood leukemia. Parents who were active in agriculture or livestock production during the assessment period completed an additional interview. Out of the parents of the cases, 16.9% were active in agriculture; of the parents of the controls, 15.6% were active. According to the national census of 2000, 18.2% of the total labor force in active in agriculture. Twenty-two pesticides were included in the exposure assessment: 2,4-D, picloram, glyphosate, benomyl, chlorothalonil, paraquat, carbofuran, mancozeb, terbufos, methamidophos, deltamethrin, methomyl, triadimefon, fluazifop, captafol, lead arsenate, malathion, dichlorvos, diuron, oxamyl, quintozene, and aldrin. A binary yes/no exposure variable was created for 14 exposure groups for exposure to any chemical in a group.

Statistical analyses: Unconditional crude and adjusted logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for exposures during 5-time windows (1 year before conception, 1st trimester of pregnancy, 2nd trimester of pregnancy, 3rd trimester of pregnancy, 1st year of life), and anytime. Models were adjusted for residence (urban, rural). Exposures were expressed as qualitative (yes, no), semiquantitative (unexposed, low exposure, high exposure) and quantitative metrics for specific pesticides and groups of pesticides. The unexposed formed the reference group in all of the analyses, except for those between the high and low exposure groups. Low correlations among the controls between variables of pesticide exposure and maternal age at conception, infectious disease of the child during the first year, the mother's and child's exposure to X-rays during pregnancy and first year of life, respectively, mother's tobacco and alcohol consumption during pregnancy, father's smoking, and history of newborn jaundice and vaccination of the child resulted in the inclusion only of the urban or rural residence in all of the logistic models controlling for unmeasured urban or rural risk

factors. The mother's X-ray exposure during pregnancy was included because of its moderate correlation with phthalide exposure.

Results

Mothers showed elevated ORs for all time intervals for all pesticides and for pesticide classes: insecticides, herbicides, and fungicides. Fathers showed elevated ORs for all pesticides during the second trimester, for insecticides during the third trimester, for herbicides during second trimester, and for fungicides in most of the time periods and anytime.

In analyses by chemical class, mothers showed elevated ORs for all time windows for organophosphates and for other pesticides (paraquat, chlorothalonil, glyphosate and other pesticides). Results by chemical class for fathers were infrequently elevated over the various time intervals and the ORs were 1.1 for other pesticides for all time intervals.

Table 3. Odds ratios (OR) and 95% confidence intervals (95% CI) for parents' exposure to pesticides to which >3 cases had been exposed through biocide action—exposed versus unexposed. (N = number of exposed cases)

Pesticide group	Fathers			Mothers		
	N	OR[a]	95% CI	N	OR[a]	95% CI
All pesticides						
1 year before conception	64	1.2	0.9–1.8	11	2.4	1.0–5.9
1st trimester of pregnancy	45	1.3	0.9–2.0	11[b]	22.0	2.8–171.5
2nd trimester of pregnancy	45	1.5	1.0–2.3	9	4.5	1.4–14.7
3rd trimester of pregnancy	36	1.2	0.8–1.9	9	2.2	0.8–5.8
1st year of life	60	1.2	0.8–1.8	10	2.0	0.8–4.8
Anytime	66	1.4	0.9–2.0	13	2.2	1.0–4.8
Insecticides						
1 year before conception	41	1.4	0.9–2.1	7	4.6	1.2–17.8
1st trimester of pregnancy	20	1.2	0.7–2.1	} 7	6.9	1.4–33.2
2nd trimester of pregnancy	21	1.2	0.7–2.0			
3rd trimester of pregnancy	19	2.2	1.2–4.1	7	3.4	1.0–11.8
1st year of life	35	1.3	0.8–2.0	6	2.9	0.8–10.5
Anytime	44	1.4	0.9–2.1	9	3.0	1.0–8.4
Herbicides						
1 year before conception	53	1.2	0.8–1.7	9	2.0	0.8–5.0
1st trimester of pregnancy	35	1.4	0.9–2.1	} 8	5.3	1.4–20
2nd trimester of pregnancy	37	1.6	1.0–2.5			
3rd trimester of pregnancy	31	1.3	0.8–2.1	7	2.3	0.8–6.8
1st year of life	53	1.3	0.8–1.9	7	1.5	0.6–4.1
Anytime	60	1.4	0.9–2.0	11	1.4	0.9–2.0
Fungicides						
1 year before conception	30	1.6	1.0–2.6	4	7.7	0.9–69.7
1st trimester of pregnancy	21	1.7	0.9–3.0	} 4	7.8	0.9–70.6
2nd trimester of pregnancy	16	1.2	0.6–2.3			
3rd trimester of pregnancy	16	1.7	0.9–3.4	4	7.8	0.9–70.6
1st year of life	28	1.5	0.9–2.5	4	2.6	0.6–11.8
Anytime	36	1.9	1.1–3.0	6	1.9	1.1–3.0

[a] Adjusted for residence (urban or rural).

[b] This OR is based on 11 exposed case mothers and 1 exposed control mother. The unadjusted OR was 21.6 (95% CI 2.8–168.0).

Discussion

The authors characterized their findings as showing an elevated risk of childhood leukemia in association with parents' occupational exposures to pesticides prior to and during pregnancy and over the first year of life. The relative excesses were considered stronger for the mothers and were found for the different types of biocides and several chemical groups, specifically: picloram, benomyl, foxim, paraquat, mancozeb, and malathion. With regard to chemical groups of pesticides, organophosphates (for the first and third trimesters) and "other" pesticides (chlorothalonil, paraquat, glyphosate and others) showed consistently higher odds ratios for the mothers than for the fathers.

Assessment and conclusion

Assessment and conclusion by applicant:

This childhood cancer case-control study showed elevated ORs for every type of pesticide exposure for mothers across 5 time periods. Glyphosate was not evaluated per se, but it was included in a category (along with chlorothalonil, paraquat, and other unspecified pesticides) that was associated with higher risk of childhood leukemia with maternal exposure. Findings for fathers were more heterogeneous and the category of pesticides that includes glyphosate was not associated with increased risk. Given that glyphosate was not studied per se, these results are not informative in terms of whether glyphosate is associated with childhood leukemia through maternal exposure.

There are a number of potential limitations that need to be considered in this study. Given that virtually every maternal exposure was associated with increased risk, it is possible, perhaps likely, that recall bias could be operative. Having experienced a childhood cancer would be a powerful stimulus to mothers to remember or report potential exposures. Given that there is no objective exposure source for participants in this study, recall bias cannot be proven. But, the pattern of elevated ORs for every exposure and time period certainly fits the paradigm for recall bias.

Selection bias seems not to be a concern as participation was similar for cases and controls.

Residual confounding would seem to be a concern. The logistic models were only adjusted for urban versus rural residence. No adjustments were made for correlated exposures. Typically, such adjustments reduce the ORs estimated for specific exposures. Of course, in analyses for pesticide classes, there is no way to differentiate the potential for a causal effect among agents in the class.

Analytic selection bias could also have resulted by setting the reference group to be those unexposed to any pesticide (see a numerical illustration in Acquavella et al. 2016). According to accepted case control theory (Rothman et al. 2008), the validity of case control studies depends on accurately estimating the exposure prevalence in the population that gave rise to the cases. Exposure prevalence cannot be estimated accurately by excluding from the reference group those with exposures other than the exposure being evaluated. This practice distorts exposure prevalences and can bias OR estimates.

In conclusion, this study is not informative for glyphosate, primarily because there were no analyses specific for glyphosate. In addition, the results are limited by the possibility for various biases that are common to pesticide case-control studies.

References

Acquavella J, Garabrant D, Marsh G, et al. Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma. Critical Rev Toxicol 2016:46(S1):28-43.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Monge P, et al. Parental occupational exposure to pesticides and risk of childhood leukemia in Costa Rica. Scand J Work Environ Health 2007; 33(4): 293-303.	**Criteria met? Y/N/?**	**Comments** This study did not address glyphosate specifically.
Study Design		
Adequate study design given study objectives	No	Specific pesticides not addressed.
Appropriate study population to address potential glyphosate-related health outcomes	Uncertain	Hard to assess the quality of the information available from study participants.
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Yes	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	No	
Adequate statistical analysis	No	
Adequate consideration of personal confounding factors	No	
Adequate consideration of potentially confounding exposures	No	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	No analyses for glyphosate per se.

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability low, case control study, potential for recall bias as indicated by the high odds ratios for virtually all pesticide groups. Lack of pesticide specific information.
Population : Reliability high, adequate inclusion of cases and control. High response rate (90%), however, number of exposed cases appear to be low for the mothers (5-11 depending on time of exposure).
Exposure assessment. : Reliability low, no chemical specific exposure information collected.
Outcome assessment: Reliability low, questionnaire with information only collected for groups of chemicals. Recall bias appears to have been an issue as indicated by the high ORs in virtually all pesticide groups for mothers.
Confounder control: Reliability high, evaluation were made for confounders including maternal age at conception, infectious disease of the child during the first year, the mother's and child's exposure during pregnancy and the first year of life, mother's tabacco and alcohol consumption during pregnancy, father's smoking, history of newborn jaundice and vaccination. Low correlations were observed resulting in the inclusion of only the urban or rural residence as well as the mother X-ray exposure during pregnancy.

Statistical methods: Reliability low, the unexposed category consisted of subjects that were unexposed to all included pesticides group which can be expected to lead to differences between the groups based on other covariates.
Reporting: Reliability high, adequate information provided on the materials and methods and results.

Overall, the reliability of the study is concluded to be low mainly due to the potential recall bias as indicated by the high ORs for virtually all pesticide groups evaluated and the lack of specific exposure information for glyphosate.

B.6.5.18.25. Supporting publications – Nordstrom, 1998

Data point	KCA 5.5-050
Report author	Nordstrom et al.
Report year	1998
Report title	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study
Reference	Brit J Cancer 1998; 77(11): 2040-2052.
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, RAR 2015
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No Conclusion AGG: The cases and controls from this paper were included in the analysis by Hardell et al. (2002). The limitations reported there also apply to the study by Nordstrom et al. 1998. It is noted that the number of glyphosate exposed cases and controls were low with only 4 exposed cases and 5 exposed controls.

Full summary of the study according to OECD format
The cases and controls from this study were included in a pooled analysis by Hardell et al. (2002). Please refer to the review of that paper.

Assessment and conclusion

Assessment and conclusion by applicant:

Please see assessment of Hardell et al. 2002.

Reference
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; 43:1043-1049.

Reliability Criteria: Epidemiology studies made by the applicant

Publication: Nordstrom M, et al. Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. Brit J Cancer 1998; 77(11): 2040-2052.	**Criteria met? Y/N/?**	**Comments** The cases and controls from this study were included in a pooled analysis by Hardell et al. (2002). Please refer to the review of that paper.
Study Design		
Adequate study design given study objectives		
Appropriate study population to address potential glyphosate-related health outcomes		
Exposure studied		
Exposure to formulations with glyphosate as a.i.		
Exposure to formulations with other a.i.		
Exposure to other farm exposures		
Study Conduct/analysis		
Adequate description of study population		
Adequate description of exposure circumstances		
Comparable participation by groups being compared		
Information provided by proxy respondents		
Adequate statistical analysis		
Adequate consideration of personal confounding factors		
Adequate consideration of potentially confounding exposures		
Overall assessment		
Reliable without restrictions		
Reliable with restrictions		
Not reliable		

Assessment and conclusion by RMS:

The cases and controls from this paper were included in the analysis by Hardell et al. (2002) which is evaluated in section B.6.5.18.16. It is however noted that for glyphosate there were only 5 exposed cases and 4 exposed controls limiting the reliability of the study.

B.6.5.18.26. Supporting publications – Orsi, 2009

Data point	KCA 5.5-051
Report author	Orsi et al.
Report year	2009
Report title	Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study
Reference	Occup Environ Med 2009; 66:291-298
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
Previous evaluation	Yes, RAR 2015
GLP/Officially recognized testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: No

	Conclusion AGG: study concluded to be of low reliability (see RMS assessment at the end of the study).

Full summary of the study according to OECD format

Orsi et al. conducted a hospital-based case-control study of lymphoid neoplasms (LN) in 6 centers in France between 2000 and 2004. **Methods:** The LN cases were incident cases, male, and diagnosed between the ages of 18 and 75 years of age. Concurrent controls were men, selected to be of the same age (± 3 years) and were recruited in the same hospital, mainly in the orthopedic and rheumatological departments. Exposures to pesticides were determined through self-reports and interviews. Four hundred and ninety-one cases (244 cases of non-Hodgkin's lymphoma (NHL), 87 of Hodgkin's lymphoma (HL), 104 of lymphoproliferative syndromes (LPSs) and 56 of multiple myeloma (MM) cases) and 456 controls were included in the analyses. **Statistical analysis:** The measure of association was the odds ratios (ORs) with 95% CIs which were estimated using unconditional logistic regression. **Results:** Positive significant associations were observed for a number of pesticide and LN subtypes, but not for NHL. There were no positive or inverse significant associations for glyphosate and any LN. **Conclusions:** The authors concluded that their results were consistent with the hypothesis that pesticides may be associated with specific LNs.

Materials and Methods

Subject selection: Orsi et al. restricted their study population to male LN patients and controls. Eligible cases were aged 20–75 years, residing in the hospital's catchment area and recently diagnosed with any a LN. All diagnoses were cytologically or histologically confirmed and reviewed by a panel of pathologists and hematologists. Patients with a history of immunosuppression or taking immunosuppressant drugs were not eligible. The controls were patients with no prior history of a LN, recruited in the same hospitals as the cases, mainly in orthopedic or rheumatology departments, matched with the cases by center and age (± 3 years). Participation was almost complete for cases (95.7%) and controls (91.2%).

Exposure assessment: Subjects were given a self-administered questionnaire for socioeconomic characteristics, familial medical history, and lifelong residential and occupational histories. The patients then underwent a face-to-face interview by trained staff using a structured standardized questionnaire eliciting personal and familial medical histories, lifestyle characteristics (smoking and alcohol, tea and coffee consumption) and outdoor leisure activities. Finally, an agricultural occupational questionnaire was administered to each patient who had worked as a farmer or gardener for at least 6 months during any period of his life. Most of the 168 subjects who were administered the specific agricultural occupational questionnaire had to be re-interviewed by telephone because the initial information was insufficient. Repeat interviews were conducted with 95 subjects (56.8%). When information on pesticides was missing or judged unreliable, experts were asked to allocate a list of chemicals that may have been used, based on the crops treated, method of spraying, period and frequency of treatment and pests targeted. Two definitions of exposure were used: possible or definite. Analyses presented in the published paper were based on possible exposures. The authors said analyses based on exposures judged to be definite, in general, produced similar or stronger associations.

Statistical analyses: ORs and 95% CIs were calculated by unconditional logistic regression for LN subgroups and LN overall. Logistic models included terms for age, center, and socioeconomic category (viz., blue or white collar). There is some lack of clarity about whether confounding by other pesticides was controlled in analyses of individual pesticides. The authors note in their methods section that in an attempt to disentangle multiple pesticide exposures, all the combinations of pesticide families associated with the LN subtype were considered two by tow and those with a p value of at least 10% were included in the logistic models. However, they also note in the discussion: "Confounding by other pesticide exposures could not be controlled since all pesticide uses were closely related to each other." Perhaps this means they were able to control in analyses based on chemical classes, but not for analyses of individual agents.

Results

There were hundreds of ORs calculated in the various analyses. For the purposes of this review, only glyphosate analyses will be detailed (table below). For all LNs combined, the OR was essentially null (OR 1.2, 95% CI 0.6, 2.1). ORs varied across LN subtypes, but none were statistically significant. Numbers of exposed cases were few.

	exposed cases	exposed controls	OR	95% CI
NHL	12	24	1.0	0.5, 2.2
HL	6	15	1.7	0.6, 5.0
LPS	4	18	0.6	0.2, 2.1
MM	5	18	2.4	0.8, 7.3
all LN	27	24	1.2	0.6, 2.1

Discussion

The authors concluded that there were numerous significant associations between pesticides and LN subtypes. They considered it notable, however, that there were no significant associations for NHL, but said their findings did not rule out a role for pesticides in the etiology on NHL. The authors did not draw any conclusions specifically for glyphosate. Glyphosate was not widely used in the study population. Considering NHL for example, only 4.9% of cases and 5.5% of controls were judged to have any exposure during their lifetimes.

Assessment and conclusion

Assessment and conclusion by applicant:

This study is not very informative about glyphosate (or other pesticides), primarily due to the small number of exposed cases, the lack of detail about exposure, and the likelihood of bias. For exposure, it is unclear whether the majority of exposed subjects used glyphosate once in their lifetimes or were more frequent users. It is also not clear whether any of those considered to be exposed were based on expert opinion and insufficient self-report. In addition, nothing is known about the exposure circumstances for glyphosate use. It is clear from the authors' description that there were problems with the agricultural questionnaire information. Most subjects were judged to need a re-interview and, of those, only 56% could be re-interviewed. In addition, there is no clarity about likely dose. Glyphosate has negligible vapor pressure and minimal skin penetration (< 1%, USEPA 2017). Biomonitoring studies of glyphosate applicators have shown median doses from farm-related applications to be approximately 10^{-4} mg/kg and the highest doses to be approximately 10^{-3} mg/kg (Neimann et al. 2015; Solomon 2016). In the largest biomonitoring study to date, 40% of farmer applicators did not have detectable traces of glyphosate in their urine (with a 1 part per billion limit of detection and 5 complete days of urine collection before (1 day) and after (4 days) the application), including 9 farmers who did applications to 100 acres or more (Acquavella et al. 2004). It is not tenable that "ever being judged to have had exposure to glyphosate" is a useful exposure metric for epidemiologic analyses. Imagine studying potential health effects related to coffee consumption or a pharmaceutical with that type of exposure metric.

Orsi et al. employed a case-control design for their study. Epidemiology textbooks describe the case control design as related to the cohort study design – the fundamental design for epidemiologic research. In fact, a case control study is best thought of as a cohort study for a source population (viz., the conceptual cohort) where the cases are detected during a specified time period and the controls are a sample of the concurrent source population that gave rise to the cases (Rothman KJ,

Greenland S, Lash T eds. 2008). Case-control studies can give valid exposure-disease estimates if, by virtue of study design or through analytic methods, they obviate issues of selection bias, recall bias, and confounding.

With respect to selection bias, a fundamental principle in case control studies is that controls need to be an unbiased sample of the source population that gave rise to the cases. That is necessary for the controls to provide a valid estimate of the source population's exposure prevalence so that the exposure odds ratio (OR) is a valid estimate of the ratio of disease rates (or relative risk (RR)) for exposed versus unexposed individuals. In case-control studies, lesser participation by controls can result in an inaccurate estimate of the exposure prevalence for the source population and result in a biased OR. Differential participation was not an issue in the present study as both case and control participation was greater than 90%. However, selection bias can result when controls, but not cases, are selected from a restricted population. In this case, selecting controls primarily from the orthopedic department could result in bias to the extent that the orthopedic patients were not representative of the general population, perhaps with respect to ability to do manual labor, history of farming, or use of pesticides.

Recall bias has been noted repeatedly as an important, intractable concern in case control studies of pesticides (Chang and Delzell 2016; Acquavella et al. 2016). In fact, the United States Agricultural Health Study – a prospective cohort study – was launched due to the perceived need to obviate recall bias with a prospective orientation between exposure assessment and disease identification (Alavanja et al. 1994). Orsi et al. (2009) tried to obviate the potential for recall bias by including non-LN patients as controls, interviewing cases and controls under similar conditions, and only telling subjects that they were in a study about the environment and health. Nonetheless, cancer is different than a fracture in terms of the self-assessment patients are likely to undergo about the cause of their illness. So, best efforts aside, it seems likely that recall bias could have been an issue for this study.

Crump (2020) conducted an analysis to try to discern whether recall bias and/or selection bias were operative in the case-control studies that are part of the glyphosate epidemiologic literature. His thesis was that since the studies that included glyphosate also included many other pesticides, one could get a measure of whether the methodology was biased by assessing the proportions of non-glyphosate ORs that were greater than the null value of 1.0. If the methodology was unbiased, the proportion of ORs greater than 1.0 for non-glyphosate exposures should be approximately 50%. For Orsi et al., (2009) the percent of non-glyphosate ORs that were greater than 1.0 was 76%. As a result, Crump (2020) concluded that studies like Orsi et al., with such a marked skew toward positive results for non-glyphosate exposures, are not reliable for determining validly exposure-disease relationships for glyphosate.

Confounding by other exposures was discussed above. Suffice it to say that it is unclear whether such confounding was addressed adequately in this study.

In conclusion, the study by Orsi et al. is not very informative with respect to glyphosate exposure. Limitations include the small number of exposed cases and the potential for recall, selection, and confounding biases.

References

Acquavella JF, Alexander BH, Mandel JS, et al. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. Environ Health Perspect 2004; 112:321-326.

Acquavella J, Garabrant D, Marsh G, et al. Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma. Critical Rev Toxicol 2016:46(S1):28-43.

Alavanja MCR, Akland G, Baird D, et al. Cancer and Noncancer Risk to Women in Agriculture and Pest Control: The Agricultural Health Study. J Occup Environ Med 1994; 36: 1247-50.

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Anal 2020; 40(4): 696-704.

Niemann L, Sieke C, Pfeil R, Solecki R. A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. J Verbr Lebensm 2015; 10:3-12.

Orsi L, Delabre L, Monnereau A, et al. Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. Occup Environ Med 2009; 66: 291–298.

Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 8, p 112.

Solomon K. Glyphosate in the general population and in applicators: a critical review of studies on exposures. Crit Rev Toxicol 2016; 46 suppl 1:21-27.

USEPA Office of Pesticide Programs. Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential, December 2017.

Reliability Criteria: Epidemiology studies made by applicant

Publication: Orsi et al. Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. Occup Environ Med 2009; 66:291-298.	**Criteria met? Y/N/?**	**Comments**
Study Design		
Adequate study design given study objectives	No	Problems with the interviews regarding pesticide exposures.
Appropriate study population to address potential glyphosate-related health outcomes	No	Inadequate control population – restricted to orthopaedic patients. Small numbers of exposed subjects.
Exposure studied		
Exposure to formulations with glyphosate as a.i.	Yes	
Exposure to formulations with other a.i.	Yes	
Exposure to other farm exposures	Uncertain	
Study Conduct/analysis		
Adequate description of study population	Yes	
Adequate description of exposure circumstances	No	
Comparable participation by groups being compared	Yes	
Information provided by proxy respondents	No	
Adequate statistical analysis	No	

Publication: Orsi et al. Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. Occup Environ Med 2009; 66:291-298.	**Criteria met? Y/N/?**	**Comments**
Adequate consideration of personal confounding factors	Yes	
Adequate consideration of potentially confounding exposures	Uncertain	
Overall assessment		
Reliable without restrictions	No	
Reliable with restrictions	No	
Not reliable	Yes	

Assessment and conclusion by RMS:

The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability low, case control study, potential for recall bias.
Population: Reliability high; high response rate (>90%), the applicant considered to the use of orthopaedic patients as controls as a limitation. However, the RMS is considers this is likely to reflect a relatively accurate representation of the general public as they do not only result from work related accidents but also in a large part due to non-work related accidents. Nevertheless, the appropriateness of using hospital controls over convenience is much debated among epidemiologists.
Exposure assessment: Reliability low, no mention of questionnaire validity in the study report, as indicated in the study report a repeat interview had to be conducted because the information was insufficient. However, for 43.2% this could not be done due to refusal, death or because subjects could no longer be contacted. Moreover, it is indicated that when information on pesticides was missing experts had to allocate a list of chemicals that may have been used. It is not clear how often this was the case. Further, the authors mention that interviews were repeated due to inconsistencies, which suggests recall bias (e.g. demand characteristics, acquiescence bias).
Outcome assessment: Reliability high, all diagnosis were cytologically or histologically confirmed by a panel of pathologists and haematologists.
Confounder control: Reliability moderate to low, ORs were adjusted for age, centre and socioeconomic category (white collar/blue collar). No adjustment for confounders such as occurrence of cancer in first degree family appears to be done. It is unclear if other pesticide exposure was taken into account as confounder.
Statistical methods: Reliability moderate, statistical methods are adequate. However, there is uncertainty regarding how and if other pesticide exposure was taken into account.
Reporting: Reliability moderate, material and methods and results for the most part adequately described. However, there is uncertainty regarding how and if other pesticide exposure was taken into account.

Overall, the reliability of the study is concluded to be low due to the limitations in the exposure assessment and the uncertainties on the confounder control.

B.6.5.18.27. Supporting publications – Studies which did not reveal an association between glyphosate and specific cancer types

Alavanja et al. 2003

1. Information on the literature article

Data point	KCA 5.5
Author	Alavanja MCR, Samanic C, Dosemeci M, et al.
Year	2003
Title	Use of Agricultural Pesticides and Prostate Cancer Risk in the Agricultural Health Study Cohort
Document No	American Journal of Epidemiology 2003; 157: 800-814.
Short description of literature article	The Agricultural Health Study (AHS) is a prospective cohort study of licensed pesticide applicators and their families. This manuscript describes an evaluation of prostate cancer incidence and related risk factors focused on 55,332 male AHS pesticide applicators from Iowa and North Carolina with no prior history of prostate cancer. The follow-up period started with initial enrollment in the study cohort (1993-1997 for various individuals) and continued through the end of 1999. The authors compared prostate cancer incidence for AHS cohort members to that for the general populations in Iowa and North Carolina. They also examined the relation between 45 common agricultural pesticides and prostate cancer within the AHS cohort. For these analyses, the authors calculated adjusted odds ratios (ORs) and 95% confidence intervals (CIs).
Short description of findings	The incidence of prostate cancer was found to be 14% higher in the AHS cohort than in the general population (incidence ratio 1.14, 95% CI 1.05, 1.24). As firmly established in the literature, having a family history of prostate cancer in a first degree relative was associated with an increased risk of prostate cancer (OR = 1.9, 95% CI 1.4, 2.7). In the various pesticide analyses, methyl bromide was associated consistently with prostate cancer. Several chlorinated pesticides were associated with prostate cancer risk only among those with a family history of prostate cancer. Glyphosate was not associated with prostate cancer risk in the various analyses.
Relevance of this literature article to the submission	This article has very limited relevance for glyphosate. Glyphosate results were not actually presented in this manuscript. Glyphosate is merely mentioned in table footnotes as having no relationship with prostate cancer risk. Given that this was more or less a fishing expedition with no a priori basis for suspecting a relationship between prostate cancer and any individual pesticide, the results might at best be considered hypothesis generating regarding methyl bromide and prostate cancer risk.
Evaluation RMS :	The publication does not provide a lot of detail on the results with glyphosate. It is merely reported that “glyphosate did not demonstrate a significant exposure-response association with prostate cancer.” Since the publication did not report an association with prostate cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

2. Alavanja et al 2012

1. Information on the literature article

Data point	KCA 5.5
Author	Alavanja MCR & Bonner M.
Year	2012
Title	Occupational Pesticide Exposures and Cancer Risk: A Review
Document No	J Toxicology and Environmental Health, Part B 2012; 15:238-263.
Short description of literature article	This is a narrative review article of the literature on pesticides and cancer risk. There are no new analyses of available studies. The authors' view is that "Chemicals in every major functional class of pesticides including insecticides, herbicide, fungicides, and fumigants have been observed to have significant associations with an array of cancer sites." That view is balanced with the observation that "…it is important to note that most pesticides have not been found to be associated with cancer in epidemiologic studies."
Short description of findings	Focusing on glyphosate, the authors mention two studies with conflicting findings for glyphosate and non-Hodgkin's lymphoma (NHL). First, they mention a Swedish case-control study by Eriksson et al. (2008) and characterize this study as showing an exposure-response relationship between glyphosate and NHL (referent odds ratio (OR) = 1.0; OR ≤ 10 days of lifetime exposure = 1.7, 95% CI 0.7, 4.1; OR > 10 days exposure = 2.4, 1.0, 5.4). The authors contrast these findings with the findings from the prospective cohort Agricultural Health Study (AHS) (of which Dr. Alavanja was the principal investigator at the time of this review article) (see De Roos et al. 2005) in which there was no association between glyphosate and NHL in any exposure category, even among those with 57 or more days of use. Alavanja and Bonner also mention multiple myeloma findings for glyphosate from De Roos et al. (2005) of an elevated, though non-significant rate ratio (RR): RR = 2.6, 95% CI 0.7, 9.4.

Relevance of this literature article to the submission	This review has no significance for the ongoing evaluation of glyphosate epidemiology. There are no original analyses and the assessment is dated. The elevated, non-significant, RR for multiple myeloma and glyphosate from the 2005 De Roos et al. publication has been found to be due to error and could not be replicated in the 2018 Andreotti et al. updated AHS glyphosate publication. The publication by Eriksson has been reviewed in detail in other parts of the dossier. Briefly, although Alavanja and Bonner report that Eriksson et al. (2008) found an exposure response relationship for glyphosate, Eriksson et al. actually said (page 1662) that they found "a tendency toward exposure response." Presumably that means that the trend analysis was not statistically significant (trend p values were not presented). In addition, in the exposure-response analysis by Eriksson et al. (2008), the ORs were not adjusted for other, potentially confounding, exposures. In other analyses in Eriksson et al. (2008), glyphosate ORs were reduced appreciably after adjustment for other exposures. Lastly, Alavanja and Bonner did not discuss the issue of recall bias in their discussion of Eriksson et al. (2008) or with respect to any other case control study referenced in their review.[6] As Crump (2020) noted with respect to Eriksson et al. (2008), 90% of the non-glyphosate ORs were greater than 1.0, suggesting the methodology was markedly skewed toward positive findings, likely due to recall bias, selection bias, or other biases. References Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst 2018: 110(5): 509-516. Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Analysis 2020; 4: 696-704. De Roos AJ, Blair A, Rusiecki JA, et al. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 113:49-54.
Evaluation RMS :	The publication concerns a review article. The reference mentioned in the publication are evaluated elsewhere in this RAR such as Eriksson et al. 2008. The study authors concluded that the available epidemiological studies for glyphosate provided inconsistent results and it was not included in the list of pesticides for which the authors concluded that the available epidemiological data suggest human carcinogenicity.

[6] A text word search for "recall bias", "recall", and "bias" did not find these terms/words mentioned in the review.

3. Andreotti et al. 2009

1. Information on the literature article

Data point	KCA 5.5
Author	Andreotti G, Freeman LB, Hou L, et al.
Year	2009
Title	Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort.
Document No	International J Cancer 2009; 124:2495-2500
Short description of literature article	This is a pancreatic case-control study within the Agricultural Health Study (AHS) cohort study. Cases (n = 93, 64 applicators, 29 spouses) were diagnosed with pancreatic cancer between enrollment (during 1993-1997) and the end of 2004. Controls were other cohort members who did not have a history of cancer (n = 82,503, 52,721 applicators, 29,782 spouses). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression as measures of association for 50 pesticides controlling for age, smoking, and diabetes. Information about pesticide use had been collected during enrollment and through a follow-up questionnaire.
Short description of findings	The authors found elevated ORs for 2 pesticides: EPTC (a thiocarbamate) and pendimethalin (a dinitroaniline). Ever use of glyphosate was not associated with pancreatic cancer (OR = 1.1, 95% CI 0.6, 1.7). Findings were similar for the highest intensity use of glyphosate (OR = 1.2, 95% CI 0.6, 2.6).
Relevance of this literature article to the submission	The relevance of this article is very limited. The findings of this study are dated. There are more recent AHS findings for glyphosate and pancreatic cancer, specifically: RR = 1.06, 95% CI 0.57, 1.97 for the highest intensity glyphosate exposure in the 2018 publication by Andreotti et al. References Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst 2018: 110(5): 509-516.
Evaluation RMS :	The study concerns an analysis of the Agricultural Health Study for associations between pesticide use and pancreatic cancer. No association was found for glyphosate with an OR of 1.1 (95% CI 0.6-1.9). Since the publication did not report an association with pancreatic cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation of the study was made.

4. Band et al. 2011

1. Information on the literature article

Data point	KCA 5.5
Author	Band PR, Abanto Z, Bert J, et al.
Year	2011
Title	Prostate Cancer Risk and Exposure to Pesticides in British Columbia Farmers
Document No	The Prostate 2011; 71: 168-183
Short description of literature article	Band et al. conducted a prostate cancer case-control study that looked to associate prostate cancer with approximately 180 pesticides or active ingredients. Cases (n = 1,516) were diagnosed with prostate cancer during the period 1983-1990. Controls (n = 4,994) were other cancer cases diagnosed during the same period, excluding lung cancers and cancers with an unknown primary site. 26% of prostate cancer cases and 20% of controls were excluded because they had worked as farmers outside of British Columbia. Proxy respondents provided information for 18% of cases and 17% of controls. Exposure assessment was indirect. Study subjects provided occupational histories and pesticide exposure was imputed based on a job-exposure matrix. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). The ORs were adjusted for alcohol consumption, cigarette years, pipe years, education level, and type of respondent.
Short description of findings	Significant associations with prostate cancer were found for numerous pesticides or active ingredients, including DDT, simazine, lindane, dichlone, dinoseb amine, malathion, endosulfan, 2,4-D, 2,4-DB, and carbaryl. The OR for ever having been exposed to glyphosate was 1.4 (95% CI 0.8, 2.3).

Relevance of this literature article to the submission	This study is essentially a hypothesis generating study of no significance to the ongoing glyphosate evaluation. There were a large number of significant positive associations with pesticides and active ingredients other than glyphosate. It is uncertain whether these associations have any validity or, instead, are due to numerous possible biases inherent in the methodology for this study. There were no findings of note for glyphosate and analyses were only detailed for ever versus never use. Far superior evidence about glyphosate and prostate cancer comes for the 2018 publication from the Agricultural Health Study (AHS) where there was no association with glyphosate overall or in any of the exposure categories (highest exposure category RR = 0.99, 95% CI 0.86, 1.13) (Andreotti et al. 2018). This highest exposure category in the 2018 AHS publication involved 109 or more cumulative days of glyphosate use. References Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst 2018: 110(5): 509-516.
Evaluation RMS:	Glyphosate is only mentioned once in the publication in the results table. Ever use of glyphosate was not significantly associated with prostate cancer (OR 1.36, 95% CI 0.83-2.25). Since the publication did not report an association with prostate cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation of the study was made.

5. Carreon et al. 2005

1. Information on the literature article

Data point	KCA 5.5
Author	Carreon T, Butler MA, Ruder AM, et al.
Year	2005
Title	Gliomas and Farm Pesticide Exposure in Women: The Upper Midwest Health Study
Document No	Environ Health Perspect 2005; 113: 546-551
Short description of literature article	Carreon et al. conducted a case control study in the US upper Midwest states to determine whether living on a farm or exposure to pesticides increased the risk of glioma for women. Cases were diagnosed and histologically confirmed during the period 1995-1997. Controls were women without a glioma diagnosis selected from driver's license records (18-64 years of age) and records of the Health Care Finance Administration (65+ years of age). Study participants were interviewed to determine their direct or indirect pesticide experience through January 1993. Proxy respondents provided information for 43% of cases, but only for 2% of controls. Odds ratios (ORs) and 95% confidence intervals were estimated by logistic regression. Analyses for specific pesticides were adjusted for age, 10-year age group, education, and any other pesticide exposure. Analyses were presented with and without proxy respondents.
Short description of findings	Only 8% of women had applied pesticides, so most exposures under study were (assumed) indirect or through laundering clothes. There were no significant associations for individual pesticides and glioma. The glyphosate OR was 0.7, (95% CI 0.4, 1.3).
Relevance of this literature article to the submission	This study has very limited relevance for glyphosate. It is doubtful that there was any appreciable glyphosate exposure for the women included in this study. It has been well demonstrated in the literature that indirect exposure to glyphosate on farms or possibly through laundering clothes is negligible (Acquavella et al. 2004). References Acquavella JF, Alexander BH, Mandel JS, et al. Glyphosate Biomonitoring for Farmer-Applicators and their Families: Results from the Farm Family Exposure Study. Environ Health Perspect 2004:112:321-326.
Evaluation RMS :	Glyphosate is only mentioned once in the publication, namely in the results table which shows that glyphosate had not association with glioma in women (OR 0.7, 95% CI 0.4-1.3 including proxy respondents ; OR 0.6, 95% CI 0.3-1.2 excluding proxy respondents). Since the publication did not report an association with glioma and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

6. Cocco et al. 2014

1. Information on the literature article

Data point	KCA 5.5
Author	Cocco P, Satta G, Dubois S, et al.

Year	2013
Title	Lymphoma risk and occupational exposure to pesticides: results of the EPILYMPH study.
Document No	Occup Environ Med. 2013; 70:91–98.
Short description of literature article	Cocco et al. (2013) reported on a lymphoma case-control analysis focused on agrochemicals. Cases and controls were recruited from six European countries during the period 1998-2003 as part of the EPILYMPH study. The study included 2,348 incident lymphoma cases and 2,462 controls. Controls were population-based in Germany and Italy. Hospital controls were recruited in the Czech Republic, France, Ireland and Spain, excluding patients with diagnoses of cancer, infectious disease, and immunodeficiency. The participation rate was 88% in cases, 81% in hospital controls, and 52% in population-based controls in Germany and Italy (see Cocco et al. 2010). Trained interviewers conducted in-person interviews. Subjects who reported having worked in agriculture were given a job-specific module inquiring in detail about tasks, kinds of crops, size of cultivated area, pests being treated, pesticides used, procedures of crop treatment, use of personal protective equipment, re-entry after application and frequency of treatment in days/year. Subjects unexposed to any pesticide were the referent category for all analyses. Exposures were classified in terms of the confidence of the classification. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for age, gender, education and study center.
Short description of findings	As the authors noted, only a few individual agrochemicals were represented by a sizable number of study subjects; too few for meaningful inference to be drawn. The authors reported an “ever exposed” glyphosate OR of 3.1 (95% CI 0.6–17.1) based on only four exposed cases and two exposed controls. Numbers were too small for an analysis that controlled for other exposures.

Relevance of this literature article to the submission	This paper has no relevance for the ongoing glyphosate analysis. As the authors noted, there were too few exposed individuals for an informative analysis of specific agrochemicals. Were numbers more robust, there would have been concerns about selection bias (esp. low participation for population controls; unrepresentativeness of hospital controls), confounding by other exposures (esp. solvent exposures found to be associated with NHL is a previous analysis of this data (Cocco et al. 2010), and recall bias. References Cocco P, t'Mannetje A, Fadda D, et al. Occupational exposure to solvents and risk of lymphoma subtypes: results from the EPILYMPH case-control study. Occup Environ Med. 2010; 67: 341–347.
Evaluation RMS	The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007). *Study design and conduct:* Reliability moderate, case-control study *Population:* Reliability moderate to low, inclusion of the study population is adequate but the number of glyphosate exposed subjects were very low (4 exposed cases and 2 controls). *Exposure assessment:* Reliability moderate, self-reported pesticide use via interviews, potential for recall bias. *Outcome assessment:* Reliability high, diagnosed lymphoma cases. *Confounder control* : Reliability low, adjustments were made for age, gender education and site of diagnosis but no adjustments were made for medical history, history of lymphoma in first degree relative or for other pesticide exposures. In particular the lack of confounder control for other pesticide use is important since the unexposed subjects were those unexposed to any pesticide. *Statistical methods :* Reliability moderate, adequate statistical methods were used but only limited adjustments for confounders were made. *Reporting* : Reliability high, key elements of the Material and methods and Results section are reported. Overall, the study is concluded to be of low reliability mainly due to the low number of glyphosate exposed subjects and the lack of adjustment for other pesticide exposures.

7. El-zaemey and Heyworth 2013

1. Information on the literature article

Data point	KCA 5.5
Author	El-Zaemey S, Heyworth J, Fritschi L.
Year	2013
Title	Noticing pesticide spray drift from agricultural pesticide application areas and breast cancer : a case-control study
Document No	Aust NZ J Public Health 2013; 37: 547-555
Short description of literature article	The authors sought to study the relationship between breast cancer and noticing pesticide spray drift from agricultural areas. They employed a case-control analysis of subjects who were participants in the Breast Cancer Environment and Employment Study (BCEES) in Western Australia. Cases (n = 1,169) were identified through the Western Australia Cancer registry. Age-matched controls (n = 1,743) were selected randomly from the Western Australian electoral role. Logistic regression was used to estimate odds ratios and 95% confidence intervals.
Short description of findings	There were no analyses for specific pesticides. The authors concluded that women who noticed spray drift had an increased risk of breast cancer.
Relevance of this literature article to the submission	This paper has no relevance for the glyphosate evaluation because there were no analyses for glyphosate or for other specific pesticides.
Evaluation RMS :	Glyphosate is only mentioned once in this publication stating that it is one of the most commonly used herbicides besides atrazine and simazine. No specific analysis is made for glyphosate exposure and therefore no conclusion can be made on the basis of this study.

8. Engel et al 2005

1. Information on the literature article

Data point	KCA 5.5
Author	Engel LS, Hill DA, Hoppin JA, et al.
Year	2005
Title	Pesticide Use and Breast Cancer Risk among Farmers' Wives in the Agricultural Health Study
Document No	Amer J Epidemiol 2005; 161: 121-135 Pest Management Science 70:869-878 (Nov 2013).
Short description of literature article	The authors investigated associations between pesticide use and breast cancer incidence among farmers' wives in the US Agricultural Health Study (AHS), a large prospective cohort study in Iowa and North Carolina. The studied population included 30,454 women with no history of breast cancer prior to cohort enrollment during the period 1993–1997. Information on pesticide use was obtained by self-administered questionnaire from the women and their husbands. The analyses included 309 incident breast cancer cases who were identified through population-based cancer registries in Iowa and North Carolina. Poisson regression was used to calculate rate ratios (RRs) and 95% confidence intervals (CIs) for individual pesticides and to control for confounding factors.
Short description of findings	Breast cancer incidence for the women included in this study was not elevated compared with that for the general populations in Iowa and North Carolina (incidence ratio 0.9, 95% CI 0.8, 1.1). There was some evidence of an association for breast cancer with use of 2,4,5-trichlorophenoxypropionic acid and possibly use of dieldrin, captan, and 2,4,5-trichlorophenoxyacetic acid, but few cases had personally used the pesticides. Glyphosate use was not associated with breast cancer incidence (RR = 0.9, 95% CI 0.7, 1.1).

Relevance of this literature article to the submission	This study has limited relevance for glyphosate. The information about pesticide use is much more limited for wives in the AHS than it is for husbands. In terms of "second-hand" exposure for spouses, biomonitoring research (Acquavella et al. 2004) has shown such exposure to be negligible for glyphosate. This study is perhaps best considered as hypothesis generating and not a study that could provide strong evidence of relationships between individual pesticides and breast cancer. References Acquavella JF, Alexander BH, Mandel JS, et al. Glyphosate Biomonitoring for Farmer-Applicators and their Families: Results from the Farm Family Exposure Study. Environ Health Perspect 2004:112:321-326.
Evaluation RMS	Glyphosate is only mentioned once in the publication, namely in the results table which shows that there is no association between glyphosate use and breast cancer (OR 0.9, 95% CI 0.7-1.1). The publication does not provide a lot of detail on the results with glyphosate. It is merely reported that "glyphosate did not demonstrate a significant exposure-response association with breast cancer." Since the publication did not report an association with breast cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

9. Flower et al. 2004

1. Information on the literature article

Data point	KCA 5.5
Author	Flower KB, Hoppin JA, Lynch CF, et al.
Year	2004
Title	Cancer Risk and Parental Pesticide Application in Children of Agricultural Health Study Participants
Document No	Environ Health Perspect 2004; 112: 631-635
Short description of literature article	As part of the Agricultural Health Study (AHS), Flower et al. sought to evaluate the risk of childhood cancers associated with parental pesticide application. Included in this study were 17,357 children (0 to 19 years) of Iowa pesticide applicators who were matched against the Iowa Cancer Registry. Fifty incident childhood cancers were identified (1975–1998). A standardized incidence ratio (SIR) was calculated to compare the observed number of childhood cancer cases identified among children of AHS participants to the expected number. The expected number of cancer cases was generated by applying age, sex, race, and time-period–specific childhood cancer rates from Iowa SEER data to the person-years contributed by the included children. The authors used logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for parental reports of pesticide application and childhood cancer risk.
Short description of findings	Childhood cancer incidence was elevated for the AHS children compared with general population rates (SIR 1.36; 95% CI, 1.03–1.79]. Risk of all lymphomas combined was also increased (SIR = 2.18; 95% CI, 1.13–4.19), as was risk of Hodgkin's lymphoma (SIR = 2.56; 95% CI, 1.06–6.14). No association was detected between frequency of parental pesticide application and childhood cancer risk. Of 16 specific pesticides used by fathers prenatally, ORs were increased for aldrin (OR = 2.66), dichlorvos (OR = 2.06), and ethyl dipropylthiocarbamate (OR = 1.91). However, these results were based on small numbers. ORs for glyphosate were 0.61 (95% CI 0.32, 1.16) for maternal use and 0.84 (95% CI 0.35, 2.34) for paternal use.

Relevance of this literature article to the submission	This study has no relevance for the ongoing glyphosate evaluation. No positive associations were found with glyphosate. More importantly, the study is based on an unproven premise that parental exposure in some way transfers over to children, either prenatally or postnatally. The authors would need to refine their study design appreciably to define exposure scenarios and exposure timings that might make sense for etiologic research. It is worth noting that glyphosate biomonitoring results for farm families show that glyphosate uptake can happen for children who are directly involved in pesticide applications (as teenage sons often are) at levels similar to those of applicators, but that indirect update by (younger male or female) children and spouses is rare (Acquavella et al. 2004). References Acquavella JF, Alexander BH, Mandel JS, et al. Glyphosate Biomonitoring for Farmer-Applicators and their Families: Results from the Farm Family Exposure Study. Environ Health Perspect 2004:112:321-326.
Evaluation RMS :	Glyphosate is only mentioned in the results table in this publication which shows that there was no association between maternal exposure (OR 0.61 ; 95% CI 0.32-1.16) or paternal exposure (OR 0.84 ; CI 0.35-2.34) and childhood cancer risk. Since the publication did not report an association with childhood cancer and since no other epidemiological studies reported an association between glyphosate and childhood cancer in general no detailed reliability evaluation was made.

10. Kachuri et al. 2013

1. Information on the literature article

Data point	KCA 5.5
Author	Kachuri L, Demers PA, Blair A, et al.
Year	2013
Title	Multiple pesticide exposures and the risk of multiple myeloma in Canadian men.
Document No	International J Cancer 2013; 133: 1846-1858.
Short description of literature article	Kachuri et al. investigated associations between pesticides and multiple myeloma (MM) in a population-based case-control study across six Canadian provinces. MM cases (n = 342) were men aged 19 years or older who were diagnosed between September 1, 1991 and December 31, 1994. MM cases were identified from provincial cancer registries except in Quebec, where cases were identified in hospitals. Controls were frequency-matched to cases by age (± 2 years) and province of residence. The control group was common to that used in the non-Hodgkin's lymphoma (NHL) case-control study by McDuffie et al. (2001). Information on pesticides used, demographic characteristics, medical and occupational history, exposure to selected chemical substances and other variables was obtained using a postal questionnaire. Subsequently, a telephone interview was arranged to gather detailed information about individual pesticide use for subjects who reported ≥ 10 hours per year of pesticide use in the postal questionnaire and a 15% random sample of the remainder. Proxy respondents provided information for 30% of the cases and 15% of the controls. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CIs). Analyses were presented with and without proxy respondents.
Short description of findings	Positive OR trends were observed for fungicides (p_{trend} = 0.04) and pesticides classified as probably carcinogenic or higher (p_{trend} = 0.03). Significantly elevated ORs were observed for those who reported using at least one carbamate pesticide (OR =1.94, 95% CI 1.16–3.25), one phenoxy herbicide (OR = 1.56, 95% CI 1.09–2.25) and ≥ 3 organochlorines (OR = 2.21, 95% CI 1.05–4.66). For specific pesticides, significant ORs were seen for carbaryl (OR = 2.71, 95% CI 1.47–5.00) and captan (OR = 2.96, 95% CI 1.40–6.24). Glyphosate ORs were 1.1 (95% CI 0.66, 1.86) for ever use, 0.7 (95% CI 0.4, 1.4) for ≤ 2 days per year of use, and 2.11 (95% CI 0.95, 4.70) for more than 2 days of use per year. The ORs cited above excluded proxy respondents and were adjusted for age, province of residence, smoking status, selected medical conditions (rheumatoid arthritis, allergies, measles, shingles, cancer) and family history of cancer.
Relevance of this literature article to the submission	This study has limited relevance for the ongoing glyphosate evaluation due to a number of important limitations in the methodology. First, none of the findings for specific pesticides was adjusted for the effects of other correlated pesticides that were also associated with MM. Given so many positive associations with MM for specific pesticides, there is likely to have been residual confounding throughout the analyses. Second, there is a marked surplus of positive pesticide/MM associations over what might be expected. It is noteworthy that this study shares a control group with the study by McDuffie et al. (2001). As Crump (2020) illustrated with respect to McDuffie's NHL study findings, the preponderance (90+%) of ORs were

> 1.0, when one would expect closer to 50% above and below the null value of 1.0. The same preponderance is apparent in Kachuri et al. Crump attributed the positive skew in the results from McDuffie et al. to be due to a combination of selection bias[7] and recall bias, but residual confounding likely is contributing as well.

The glyphosate OR of 2.11 (95% CI 0.95, 4.70) for more than 2 days per year of use (for an unspecified number of years), is best considered in the context of the most recent MM findings for glyphosate from the Agricultural Health Study (AHS) (Andreotti et al. 2018). In the table below, MM results for glyphosate are taken from the supplemental Andreotti et al. (2018) tables where rate ratios (RRs) were given by cumulative days of glyphosate use. These RRs were adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, and 2,4-D.

Exposure	# cases	RR	95% CI
No glyphosate	30	1.0	Reference
1 to 14 days	23	0.9	0.5, 1.6
14 to 39 days	22	0.8	0.4, 1.5
39 to 108 days	22	0.9	0.5, 1.7
≥ 109 days	22	0.8	0.4, 1.5

Given the concerns about bias in Kachuri et al. noted above and the lack of any association for MM with glyphosate in the AHS for exposure frequencies of 109+ days, the results for Kachuri et al. for glyphosate are of questionable validity.

References

Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst 2018: 110(5): 509-516. [and supplemental tables]

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Analysis 2020; 4: 696-704.

McDuffie HH, Pahwa P, McLaughlin JR, et al. Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev. 2001; 10:1155–1163.

Evaluation RMS: The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007).

Study design and conduct: Reliability moderate, case-control study
Population: Reliability high, cases identified from cancer registries
Exposure assessment: Reliability moderate to low, information via questionnaire and subsequent telephone interview. There is a potential for

[7] In McDuffie et al., case participation was 67% and control participation was 48%. In Kachuri et al., case participation was 58% and control participation was 48%.

recall bias as is indicated by the high ORs (>1) for all pesticides analysed. Participants were send a list of pesticides a week before the interview.
Outcome assessment: Reliability high, tumour tissue slides for 125 out of 342 MM cases were evaluated by a pathologist to confirm the diagnosis. A higher number of tumour slides could not be obtained due to changes in hospital policies regarding supplying pathological material. Controls were selected by health insurance records, random digit dialling or voters lists.
Confounder control: Reliability moderate, adjustments were made for age, province of residence, use of a proxy respondent, personal and family history and smoking history. No adjustments were made for exposure to other pesticides.
Statistical methods: Reliability moderate, adequate statistical methods used but not all relevant confounders considered.
Reporting : Reliability high, key elements of the Material and Method and Results section were reported.

Overall, the study is considered to be of moderate reliability (Klimisch Score 2).

Ever use of glyphosate was not associated with multiple myeloma (OR 1.11, 95% CI 0.66-1.86). The authors did report a nearly significant association when considering >2 days/year of glyphosate use (OR 2.11, 95 CI 0.95-4.70). However, it is noted that there was a fairly low number of exposed cases (n=10).

11. Karunanayake et al. 2011

1. Information on the literature article

Data point	KCA 5.5
Author	Karunanayake CP, Spinelli JJ, McLaughlin JR, et al.
Year	2012
Title	Hodgkin Lymphoma and Pesticide Exposure in Men: A Canadian Case-Control study
Document No	J Agromedicine 2012; 17:30-39.
Short description of literature article	The authors investigated associations between specific pesticides and Hodgkin lymphoma through a population-based, case-control study in six regions of Canada. The study was restricted to males and included 316 cases, identified during the period September 1991 through December 1994, and 1,506 controls. Cases (68%) participated to a much higher degree than controls (48%). Data were collected by a mailed questionnaire followed by a telephone interview to obtain pesticide exposure information for those reporting ≥ 10 hours per year of pesticide exposure. Conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) as measures of association.
Short description of findings	Several factors were identified as predictors for increased Hodgkin lymphoma risk: family history of cancer, exposure to the insecticide chlorpyrifos (OR = 1.19, 95% CI 1.03, 1.37, and a previous diagnosis of acne or shingles. The OR for glyphosate indicated no association with Hodgkin lymphoma (OR = 0.99, 95% CI 0.62, 1.56).

Relevance of this literature article to the submission	This study has very limited relevance for glyphosate. Overall, the exposure assessment is superficial, and the investigators really know very little about the likely extent of exposure to the various pesticides for study participants. As with many pesticide case-control studies, there are concerns about recall bias. In addition, given the marked disparity in participation by cases (68%) and controls (48%), selection bias would be an important concern.
Evaluation RMS	The study was evaluated for its reliability using some of the recommendations made in the Scientific Opinion of the PPR Panel on the follow-up of the findings of the External Scientific Report 'Literature review of epidemiological studies linking exposure to pesticides and health effects' (EFSA Journal 2017 ; 15(10):5007). *Study design and conduct:* Reliability moderate, case-control study *Population:* Reliability moderate, cases were identified through cancer registries and hospitals. Controls were selected from health insurance records, computerized telephone listing or voter's lists. The response rate was a lot higher for the cases than the controls *Exposure assessment:* Reliability moderate, first stage self-administered questionnaire followed by telephone interview for detailed pesticide exposure information. *Outcome assessment:* Reliability high, cases were confirmed by pathologist *Confounder control*: Reliability high, adjustments were made for age, province of residence, exposure to four pesticides (2,4-D, mecoprop, malathion and diazinon) that were highly corelated with exposure days of another pesticide (chlorpyrifos). *Statistical methods:* Reliability high, adequate statistical methods were applied. *Reporting*: Reliability high, key element of the Materials and Methods and Results section are sufficiently described. Overall, the study is concluded to be of moderate reliability (Klimisch Score 2). In the study glyphosate was not associated with Hodgkin Lymphoma (ORadj 0.99 95% CI 0.62-1.56)

12. Koutros et al. 2011

1. Information on the literature article

Data point	KCA 5.5
Author	Koutros S, Andreotti G, Berndt SI, et al.
Year	2011
Title	Xenobiotic-metabolizing gene variants, pesticide use, and the risk of prostate cancer
Document No	Pharmacogenetics and Genomics 2011; 21: 615-623
Short description of literature article	The authors evaluated pesticide–SNP (single nucleotide polymorphisms) in xenobiotic metabolic enzyme (XME) genes among 776 prostate cancer cases and 1444 controls in the Agricultural Health Study (AHS). Cases and controls reported pesticide exposure information on enrollment (1993-1997) and follow-up questionnaires and had to have contributed a sample of buccal cells that had sufficient quality DNA for analysis. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

	Multiplicative SNP–pesticide interactions were calculated using a likelihood ratio test.
Short description of findings	The most consistent positive results in the study were for petroleum distillates. Analyses of specific pesticides and prostate cancer risk showed significant trends for 6 of 45 pesticides; 5 of the 6 significant trends were inverse, (implausibly) consistent with a protective effect. The ORs for glyphosate were 0.9 (95% CI 0.7, 1.1) for low exposure and 0.9 (95% CI 0.7, 1.2) for high exposure. The number of pesticide-XME interactions among thousands of analyses (i.e., 1,913 SNPs in 149 candidate genes evaluated for 45 pesticides) was consistent with the number expected by chance alone.
Relevance of this literature article to the submission	This study has no relevance for glyphosate. Glyphosate was not found to be related to prostate cancer risk. Glyphosate is not metabolized to any appreciable extent (USEPA 2017). Inhalation and dermal absorption are minimal, and any systemic dose received is excreted rapidly primarily as the parent compound. References USEPA Office of Pesticide Programs. Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential. December 12, 2017
Evaluation RMS :	Glyphosate is only mentioned in the results tables of this publication which indicates that glyphosate does not have an effect on prostate cancer. Since the publication did not report an association with prostate cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

13. Landgren et al. 2009

1. Information on the literature article

Data point	KCA 5.5
Author	Landgren O, Kyle RA, Hoppin JA, et al.
Year	2009
Title	Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study
Document No	Blood 2009; 113:6386-6391.
Short description of literature article	Landgren et al. (2009) evaluated the prevalence of monoclonal gammopathy of undetermined significance (MGUS) (a condition that is sometimes a precursor to multiple myeloma) among a sample of 678 Agricultural Health Study (AHS) participants selected based on their lifetime organophosphate use. Subjects had completed all three phases of the AHS questionnaires, enrolled into a neurobehavioral study nested within the AHS cohort, and provided serum for analysis. MGUS prevalence for this sample was compared to that for the general population of Olmsted County, Minnesota (due to availability of Mayo Clinic MGUS screening data). Odds ratios (ORs) and 95% confidence intervals (CIs) for associations between MGUS prevalence and pesticide exposures were assessed by logistic regression models adjusted for age and education level.

Short description of findings	Compared with men from Minnesota, the AHS participants' rate ratio (RR) for prevalence of MGUS was 1.9 (95% CI 1.3, 2.7). The prevalence OR for MGUS for glyphosate users versus non-users, adjusted for age and education level, was 0.5 (95% CI 0.2–1.0). None of the herbicides studied showed a strong association with MGUS. The authors concluded that the elevated prevalence of MGUS among pesticide applicators supports the hypothesis that specific pesticides are causatively linked to myelomagenesis.
Relevance of this literature article to the submission	This study has no relevance for the ongoing evaluation of glyphosate. This was a small exploratory study. Findings for individual pesticides were hampered by the lack of adjustment for other pesticides in pesticide-specific analyses, the cross-sectional nature of the study, and the implied speculative hypothesis underlying the analysis (that pesticides might cause multiple myeloma by causing MGUS first).
Evaluation RMS :	This study found no association between glyphosate use and monoclonal gammopathy of undetermined significance (MGUS). However, since one public literature study in Vk*MYC mice did indicate a potential effect (Wang et al. 2019, B.6.5.18.9) a detailed assessment of the reliability of the current study was made. *Study design and conduct:* Reliability high, prospective cohort study *Population:* Reliability high – anybody seeking licenses for pesticide spraying between 1993 and 1997 was invited. No potential for selection bias. *Exposure assessment:* Reliability high – pesticide use was determined at enrolment (no issues for recall bias) and in a follow-up questionnaire after 5 years. *Outcome assessment:* Reliability high - MGUS was determined using serum samples for the study population. *Statistical methods:* Reliability high, Associations of MGUS prevalence with pesticide exposures, demographics, and subject characteristics were assessed in logistic regression models adjusted for age and education level. For every significant association between a specific pesticide and MGUS, the authors evaluated the 5 most highly correlated pesticides with the pesticide of interest as a potential source of confounding. The authors also assessed the potential confounding effect of other pesticides that had a significant association with MGUS. *Reporting*: Reliability high, key elements of the Materials and Methods and Results section are reported in sufficient detail. Overall, the study is concluded to be of high reliability. No association between glyphosate and MGUS was reported (OR 0.5, 95% CI 0.2-1.0).

14. Lee et al. 2004

1. Information on the literature article

Data point	KCA 5.5
Author	Lee WJ, Lijinsky W, Heinemann EF, et al.
Year	2004
Title	Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus
Document No	Society of Chemical Industry (2013). Pest Management Science 70:869-878 (Nov 2013).

Short description of literature article	Lee et al. reported on a population-based case-control study in eastern Nebraska. Telephone interviews were conducted with men and women diagnosed with adenocarcinoma of the stomach (n = 170) or oesophagus (n = 137) between 1988 and 1993 and concurrent controls (n = 502) randomly selected from the same geographical area. Because of the poor prognosis for the cancers, proxy interviews were conducted with 80% of stomach and 76% of oesophagus cancer cases. Proxy interviews were conducted for 61% of controls. The interviews focused on personal and family medical history, residential history, occupational history, and pesticide use. Logistic regression was used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for farming and for use of individual and chemical classes of insecticides and herbicides, including pesticides classified as nitrosatable (able to form N-nitroso compounds on reaction with nitrite).
Short description of findings	There were no associations for either cancer with ever-use of insecticides or herbicides. Individual pesticides, including nitrosatable pesticides, were not significantly associated with risk. The ORs for glyphosate were 0.8 (95% CI 0.4, 1.5) for stomach cancer and 0.7 (95% CI 0.3, 1.4) for oesophageal cancer.
Relevance of this literature article to the submission	This study has no relevance for the ongoing glyphosate evaluation. No associations were seen for glyphosate and the focus of the study was on nitrosatable pesticides, which do not include glyphosate. This study was obviously poorly conceived. One would not normally initiate a study of occupational exposures where 75+% of the information for cases would have to come from secondary respondents. The quality of the exposure information was likely very poor.
Evaluation RMS:	Glyphosate exposure was not associated with stomach cancer (OR 0.8, 95% CI 0.4-1.5) or with oesophageal cancer (OR 0.7, 95% CI 0.3-1.4). Since the publication did not report an association with stomach and oesophageal cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

15. Mink et al. 2012

1. Information on the literature article

Data point	KCA 5.5
Author	Mink PJ, Mandel JS, Sceurman BK, Lundin JI.
Year	2012
Title	Epidemiologic studies of glyphosate and cancer: A review
Document No	Regulator Toxicology and Pharmacology 2012; 63: 440-452

Short description of literature article	Mink et al. conducted a systematic review of the epidemiologic studies for glyphosate. They searched multiple databases and identified 7 cohort study publications (all from the Agricultural Health Study (AHS)) and 14 case control study publications.
Short description of findings	The authors found no consistent pattern of positive associations for glyphosate with total cancer or for specific cancers. They also discussed the

	implications of biomonitoring data for glyphosate and advised against using generic algorithms for exposure-response analyses.
Relevance of this literature article to the submission	This review is dated and has limited relevance for the ongoing glyphosate evaluation. Several more recent publications have appeared that provide updated findings for glyphosate from the AHS (Andreotti et al. 2018) and insights into sources of bias in the non-Hodgkin's lymphoma case control studies (Crump 2020).
	References Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst 2018: 110(5): 509-516. Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Analysis 2020; 4: 696-704.
Evaluation RMS :	The publication is a review paper which does not provide any new information not already reported elsewhere. It is noted by the RMS that this publication was funded by Industry.

16. Multigner et al 2008

1. Information on the literature article

Data point	KCA 5.5
Author	Multigner L, Ndong JR, Oliva A, Blanchet P.
Year	2008
Title	Environmental pollutants and prostate cancer: Epidemiological data [in French with an English abstract]
Document No	Gynecologie Obstetrique & Fertilite 2008; 36: 848–856
Short description of literature article	Multigner and colleagues published a narrative review article about environmental exposures and prostate cancer. They cited 1 prostate cancer finding for glyphosate from the Agricultural Health Study (AHS) (De Roos et al. 2005).
Short description of findings	The authors' overall conclusion was that, with few exceptions, there are no demonstrations in the literature of a significant association between exposure to pesticides or a chemical family of pesticides and prostate cancer. Methyl bromide was an exception based on the AHS findings by Alavanja et al. (2003). The authors noted the prostate cancer glyphosate rate ratio (RR) and 95% confidence interval (CI) from the AHS in 2005 of 1.1 (95% CI 0.9, 1.3) (De Roos et al. 2005), but did not reach any specific conclusions for glyphosate.

Relevance of this literature article to the submission	This study has no relevance for the ongoing evaluation of glyphosate. There are recent AHS findings for prostate cancer and glyphosate that are much more robust than those cited in this review article (see updated results from Andreotti et al. 2018 in the following table).

Exposure	# cases	RR	95% CI
No glyphosate	579	1.0	Reference
Quartile 1	571	1.0	0.9, 1.1
Quartile 2	564	1.0	0.8, 1.1
Quartile 3	559	1.0	0.9, 1.2
Quartile 4	571	1.0	0.9, 1.1

References

Alavanja MC, Samanic C, Dosemeci M, et al. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. Am J Epidemiol 2003; 157: 800–14.

Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509–516.

De Roos AJ, Blair A, Rusiecki JA, et al. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. Environ Health Perspect 2005; 113:49-54.

Evaluation RMS :	Except for the abstract the publication is entirely in French. However, glyphosate is only reported once in the publication in the results table which show that there is no association between glyphosate and prostate cancer (OR 1.1, 95 CI 0.9-1.3). Since the publication did not report an association with prostate cancer and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

17. Pahwa et al. 2011

1. Information on the literature article

Data point	KCA 5.5
Author	Pahwa P, Karunanayake C.P, Dosman J.A, Spinelli J.J, McLaughlin J.R. and Cross-Canada Group
Year	2011
Title	Soft-tissue sarcoma and pesticide exposure in men.
Document No	JOEM, 2011: 53(11): 1279-1286
Short description of literature article	Pahwa et al. (2012) reported on a Canadian, multi-center case-control study to evaluate possible relationships between pesticide exposures and soft tissue sarcoma (STS). There were 357 cases diagnosed with STS. The study design aimed to have at least one control participant matched for each case according to age and province of residence. However, difficulties occurred in recruiting controls in the older age groups (eg, 69 years and older); thus, the total number of control participants was 1506. The response rate was higher in cases (60.8%) than in controls (48%). Incident cases were men aged 19 years or older, with first diagnosis of STS from September 1, 1991, to December 31, 1994. Cases were ascertained from provincial cancer

	registries, except in Quebec, where hospital ascertainment was used. Data were collected in two stages: by self-administered postal questionnaire in stage 1; and detailed pesticide exposure information was collected via telephone interview in stage 2. Results were reported as odds ratios (OR) and 95% confidence intervals (CI) derived from conditional logistic regression analyses. Conditional logistic regression models were utilized to conduct bivariable (to select variables for multivariable model) and multivariable analyses by adjusting for age group and province of residence (matching variables). Adjustments were made statistically significant medical history variables (history of measles, rheumatoid arthritis, mononucleosis, whooping cough, and a positive family history of cancer in a first-degree relative) and with strata for the variables of age group and province of residence. No adjustment was made for exposure to other pesticides.
Short description of findings	No association between the use of glyphosate and STS was observed (OR 0.9, 95% CI 0.58-1.40).
Relevance of this literature article to the submission	This study has limited relevance for the ongoing evaluation of glyphosate since no effect on STS was observed.
Evaluation RMS :	No association was observed between glyphosate exposure and STS. Since the publication did not report an association with soft-tissue sarcoma and since no other epidemiological studies reported such a link no detailed reliability evaluation was made.

18. Pahwa et al. 2012

1. Information on the literature article

Data point	KCA 5.5
Author	Pahwa P, Karunanayake CP, Dosman JA, et al.
Year	2012
Title	Multiple Myeloma and Exposure to Pesticides: A Canadian Case-Control Study
Document No	J Agromedicine 2012; 17(1): 40-50
Short description of literature article	Pahwa et al. (2012) reported on a Canadian, multi-center case-control study to evaluate possible relationships between pesticide exposures and multiple myeloma (MM). The analysis by Parwa et al. was updated within a year by Kachuri et al. (2013). The updated analysis by Kachuri et al. refined the analysis by Parwa et al. by excluding the 10% of controls (149 of 1,506) who did not have an age match with a MM case, adjusting odds ratios for smoking which was associated with MM, and providing analyses including and excluding proxy respondents (30% for cases and 15% for controls). Please see the review for Kachuri et al. for a critique of the trans-Canada case-control study of MM.
Short description of findings	See review of Kachuri et al. 2013.
Relevance of this literature article to the submission	None. Superseded by Kachuri et al. 2013.
	References Kachuri L, Demers PA, Blair A, et al. Multiple pesticide exposures and the risk of multiple myeloma in Canadian men. Int J Cancer 2013; 133:1846–1858.
Evaluation RMS :	The case-control study reported in this publication is also reported in the publication by Kachuri, 2013. Similar to the Kachuri, 2013 study no significant association between glyphosate and multiple myeloma was observed (OR 1.22, 95% CI 0.77-1.93).

19. Schinasi and Leon 2014

1. Information on the literature article

Data point	KCA 5.5
Author	Schinasi L, Leon ME.
Year	2014
Title	Non-Hodgkin Lymphoma and Occupational Exposure to Agricultural Pesticide Chemical Groups and Active Ingredients: A Systematic Review and Meta-Analysis
Document No	Int. J. Environ. Res. Public Health 2014; 11:4449-4527
Short description of literature article	Schinasi and Leon conducted a systematic review and a series of meta-analyses of epidemiologic studies that addressed the possible relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to agricultural pesticides. Findings for NHL and 21 pesticide chemical groups and 80 active ingredients were extracted from 44 papers. Random effects meta-analyses were used to aggregate the results across studies.
Short description of findings	The authors concluded that phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and the active ingredient lindane were positively associated with NHL. For glyphosate, they calculated a meta relative risk (mRR) of 1.5 (95% confidence interval (CI) 1.1, 2.0) for one day or more of use in a lifetime.
Relevance of this literature article to the submission	This publication has very limited relevance for the evaluation of glyphosate and NHL. First, there were data extraction errors by Schinasi & Leon that were identified in a subsequent meta-analysis by Chang and Delzell (Chang & Delzell 2016). Specifically, Schinasi and Leon's methodology called for the most adjusted estimates from each study to be incorporated in their meta-analysis calculations. However, that was not the case for some glyphosate findings included in their meta-analysis. Chang & Delzell's replicated Schinasi and Leon's intended methodology and calculated a lower mRR of 1.3 (95% CI 1.0, 1.6). Second, meta-analyses can become out of date when there are new findings. Since the publication by Schinasi and Leon, a substantial glyphosate update was published by investigators from the Agricultural Health Study (AHS) (Andreotti et al. 2018). Incorporating these results gives a mRR of 1.1 (95% CI 0.9, 1.5) (see table 1).[8]

Table 1 - Updated glyphosate NHL meta-analysis calculation

Author	Year	RR	95% CI	weight
McDuffie et al.	2001	1.20	0.83, 1.74	23.19%
Hardell et al.	2002	1.85	0.55, 6.20	4.57%
DeRoos et al.	2003	1.6	0.9, 2.8	14.79%
Eriksson et al.	2008	1.51	0.77, 2.94	11.85%
Orsi et al.	2009	1.0	0.5, 2.2	10.25%
Andreotti et al.	2018	0.85	0.73, 1.00	35.36%
Meta RR		1.1	0.9, 1,5	

[8] Random effects meta-analysis calculations were done using Stata v 14.2 and validated with Episheet (www.krothman.org/episheet xls).

Third, meta-analysis involves taking a weighted average of results as they are in the literature. Meta-analysis does not correct for bias in the results being averaged. As Crump (2020) has illustrated, the studies of McDuffie et al. (2001), Hardell et al. (2002), and Eriksson et al. (2008) showed odds ratios (ORs) greater than the null value of 1.0 for 90% or more of their ORs for non-glyphosate pesticides. Therefore, Crump concluded that the findings from these studies were skewed positive, likely due to recall and selection biases, and were not reliable for glyphosate or for the other pesticides included in those studies. Accordingly, a mRR that includes these studies will be biased. In addition, as Greenland and O'Rourke (2008) note in their textbook chapter on meta-analysis, when the variation of results across studies is due to systematic factors (e.g., case recall bias, selection bias, uncontrolled confounding), the assumptions underlying random effects models are violated. As a result, related p values and 95% CIs are not valid.

Lastly, if one wants the best evidence about glyphosate's possible association with NHL, it makes more sense to look at the results for the most frequently exposure population(s), not a weighted average of results based on one day or more of exposure during a lifetime. Results for the most frequently exposed population has been published from the AHS by Andreotti et al. (see table 2 below based on the supplemental tables from Andreotti et al. 2018).

Table 2 - NHL Results by Days of Use from the AHS

Exposure	# cases	RR	95% CI
No glyphosate	135	1.0	Reference
1 to 14 days	103	0.8	0.6, 1.0
14 to 39 days	117	0.9	0.7, 1.1
39 to 108 days	107	0.9	0.6, 1.1
≥ 109 days	116	0.8	0.6, 1.1

These results for applicators with weeks and months of glyphosate use are more informative than a meta-analysis based on 1 day of glyphosate use or more during a lifetime.

References

Andreotti G, Koutros S, Hofmann JN, et al. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509–516. [and supplemental tables]

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402–428.

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Analysis 2020; 4: 696-704.

Greenland S, O'Rourke K. In Rothman KJ, Greenland S, Lash TL. 2008. Modern epidemiology. 3rd ed. Philadelphia (PA): Wolters Kluwer/Lippincott, Williams & Wilkins, chapter 33.

Evaluation	The publication provides a meta-analysis of epidemiological studies on occupational exposure to agricultural pesticides and Non-Hodgkin lymphoma. The report is considered outdated as new studies are now available. Refer to section B.6.5.18.28 for a full evaluation.

20. Weichental et al. 2010

1. Information on the literature article

Data point	KCA 5.5
Author	Weichenthal S, Moase C, Chan P.
Year	2010
Title	A Review of Pesticide Exposure and Cancer Incidence in the Agricultural Health Study Cohort
Document No	Environ Health Perspect 2010; 118: 1117-1125
Short description of literature article	Weichenthal et al. conducted a narrative review of 28 publications from the Agricultural Health Study (AHS). They identified studies from the publication list on the AHS website and through a PubMed literature search through March 2009.
Short description of findings	The (Canadian) authors concluded that there were positive associations with cancer(s) incidence for 12 pesticides registered in Canada. Glyphosate was not one of the 12 pesticides.
Relevance of this literature article to the submission	This narrative review has nothing to add to the ongoing assessment for glyphosate. The findings are dated and there were no new analyses or findings for glyphosate that were considered especially noteworthy by the authors.
Evaluation RMS :	The study is a review article of the Agricultural Health Study cohort which are already evaluated elsewhere in this RAR. The authors conclude that the is no significant increase in cancer incidences for glyphosate. However, it is noted that this study is now dated as new information is available.

B.6.5.18.28. *Public literature – Non-Hodgkin lymphoma and occupational exposure to agricultural pesticides (study for which the RMS requested a summary in order to justify the categorization)*

1. **Information on the study**

Data point:	CA 5.5
Report author	Schinasi L. and Leon ME.
Report year	2014
Report title	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis
Document source	International Journal of Environmental Research and Public Health (2014), Vol. 11, No. 4, pp. 4449-4527

Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. **Full summary of the study according to OECD format**

The authors conducted a systematic review and a meta-analysis of the extant epidemiologic research as of 2014 on non-Hodgkin lymphoma (NHL) and occupational exposure to agricultural pesticides. Estimates of associations of NHL with 21 pesticide-chemical groups and 80 active ingredients were extracted from 44 papers. Meta-relative risks (mRRs) were calculated with random-effects models. These analyses showed that phenoxy herbicides, glyphosate, carbamate insecticides, organophosphorus insecticides and the active ingredient lindane, an organochlorine insecticide, were positively associated with NHL. In a handful of papers, associations between pesticides and NHL subtypes were reported; B cell lymphoma was positively associated with phenoxy herbicides and the organophosphorus herbicide glyphosate. Diffuse large B-cell lymphoma was positively associated with phenoxy herbicide exposure.

Materials and methods

The authors used standard procedures to identify all relevant articles. Their search used combinations of the following words: occupational exposure, pesticides, insecticides, herbicides, fungicides, neoplasms, cancer, lymphomas, non-Hodgkin lymphoma, cancer mortality, agricultural workers' diseases/chemically induced, and humans. Searches employed PubMed and Web of Science databases.

From every relevant paper, the authors extracted an effect estimate for each active ingredient and/or chemical group with NHL, and when available, for associations with subtypes of NHL. For related papers that examined the same exposure/outcome association, the authors used the results from the most updated analysis. As the various papers used different confounder adjustment sets, the authors focused on the most adjusted effect estimate in order to minimize confounding to the extent possible in each paper.

The statistical analysis involved the calculation of a meta-relative risk (mRR) estimates and 95% confidence intervals (CIs) using random effect models. The authors also presented forest plots for meta-analyses to which five or more papers contributed.

Results [focusing on glyphosate]

The authors reported a positive association between glyphosate and NHL overall based on 6 studies (mRR = 1.5, 95% CI 1.1-2.0) and for glyphosate and B cell lymphoma based on 2 studies (mRR = 2.0, 95% CI 1.1-3.6).

Conclusion

The authors concluded that their results indicated positive associations between NHL and glyphosate and several other pesticides. Among the few papers that reported results for NHL subtypes, the authors concluded that there were stronger associations between certain chemicals including glyphosate and B cell lymphomas.

3. **Assessment and conclusion**

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: This paper concerns a meta-analysis where the results were taken from available studies at face value. The authors had no way to correct for recall bias, confounding,

etc. As the meta-RRs of the studies included are in error the meta-analyses are also in error. The study is considered unreliable.

Further points for clarification:
This publication has very limited relevance for the evaluation of glyphosate and NHL. First, there were data extraction errors by Schinasi & Leon that were identified in a subsequent meta-analysis by Chang and Delzell (Chang & Delzell, 2016). Specifically, Schinasi and Leon's methodology called for the most adjusted estimates from each study to be incorporated in their meta-analysis calculations. However, that was not the case for some glyphosate findings included in their meta-analysis. Chang & Delzell replicated Schinasi and Leon's intended methodology and calculated a lower mRR of 1.3 (95% CI 1.0-1.6).

Second, meta-analyses can become out of date when there are new findings. Since the publication by Schinasi and Leon, a substantial glyphosate update was published by investigators from the Agricultural Health Study (AHS) (Andreotti *et al*. 2018). Incorporating these results gives an mRR of 1.1 (95% CI 0.9-1.5) (see Table 1).[9]

Table 1. Updated glyphosate NHL meta-analysis calculation

Author	Year	RR	95% CI	weight
McDuffie *et al*.	2001	1.20	0.83-1.74	23.19%
Hardell *et al*.	2002	1.85	0.55-6.20	4.57%
DeRoos *et al*.	2003	1.6	0.9-2.8	14.79%
Eriksson *et al*.	2008	1.51	0.77-2.94	11.85%
Orsi *et al*.	2009	1.0	0.5-2.2	10.25%
Andreotti *et al*.	2018	0.85	0.73-1.00	35.36%
Meta RR		1.1	0.9-1.5	

Third, meta-analysis involves taking a weighted average of results as they are in the literature. Meta-analysis does not correct for bias in the results being averaged. As Crump (2020) has illustrated, the studies of McDuffie *et al*. (2001), Hardell *et al*. (2002), and Eriksson *et al*. (2008) showed odds ratios (ORs) greater than the null value of 1.0 for 90% or more of their ORs for non-glyphosate pesticides. Therefore, Crump concluded that the findings from these studies were skewed positive, likely due to recall and selection biases, and were not reliable for glyphosate or for the other pesticides included in those studies. Accordingly, an mRR that includes these studies will be biased.

Lastly, if one wants the best evidence about glyphosate's possible association with NHL, it makes more sense to look at the results for the most frequently exposed population(s), not a weighted average of results based on ever using glyphosate (even for as little as one day during a lifetime). Results for the most frequently exposed population has been published from the AHS by Andreotti *et al*. (see Table 2 below based on the supplemental tables from Andreotti *et al*. 2018).

Table 2. NHL results by days of use from the AHS

Exposure	# cases	RR	95% CI
No glyphosate	135	1.0	Reference
1 to 14 days	103	0.8	0.6-1.0
14 to 39 days	117	0.9	0.7-1.1
39 to 108 days	107	0.9	0.6-1.1
≥109 days	116	0.8	0.6-1.1

These results for applicators with weeks and months of glyphosate use are more informative than a meta-analysis based on 1 day of glyphosate use or more during a lifetime. It is also noteworthy that the prospective nature of the AHS cohort study precluded case recall bias, of concern in the case control-studies, and that the AHS investigators did a thorough job of controlling for confounding in their RR estimates – in contrast to the sparse control of confounding factors in the case-control studies.

[9] Random-effects meta-analysis calculations were done using Stata v 14.2 and validated with Episheet (www.krothman.org/episheet xls).

References

Andreotti G, Koutros S, Hofmann JN, *et al*. Glyphosate use and cancer incidence in the Agricultural Health Study. J Natl Cancer Inst 2018; 110(5): 509-516.

Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. J Environ Sci Health, Part B 2016; 51 (6): 402-428.

Crump K. The Potential Effects of Recall Bias and Selection Bias on the Epidemiological Evidence for the Carcinogenicity of Glyphosate. Risk Analysis 2020; 4: 696-704.

Assessment and conclusion by RMS:

In this open-literature study the results of a meta-analysis of six epidemiological studies on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides are published (based on McDuffie et al., 2001, Hardell et al., 2002, DeRoos et al., 2003&2005, Eriksson et al., 2008 and Orsi et al., 2009). Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL (not shown in the summary above). For glyphosate, the study authors calculated an increased meta relative risk (mRR) of 1.5 (95%-CI 1.1-2.0) for one day or more of use of glyphosate in a lifetime. However, there were data extraction errors by the study authors that were identified in a subsequent meta-analysis by IARC working groups and by Chang and Delzell (2016), which were also highlighted by the applicant. Moreover, a possible causal relationship was not discussed by the study authors. In addition, there is a more recent meta-analysis available using AHS data with extending cancer incidence follow-up through 2012 in North Carolina and 2013 in Iowa and incorporating additional exposure information from a follow-up questionnaire (Andreotti et al. 2018 (refer to B.6.5.18.10)).

In addition, the following shortcomings can be defined: meta-analysis mixes different study designs, biases cannot be controlled through the meta-analysis because those biases are of different natures and weight amongst the studies that constitute the meta-analysis and the authors did not make an effort to include studies published in languages other than English.

As the information from this study, together with extended data from a follow-up was evaluated by Andreotti (2018), the study by Andreotti is considered further in the risk assessment and this study by Schinasi (2014) is considered to be supportive.

B.6.5.18.29. *Public literature – Systematic review and meta-analysis of glyphosate exposure and risk of lymphohmatopoietic cancers (study for which the RMS requested a summary in order to justify the categorization)*

1. Information on the study

Data point:	CA 5.9.4
Report author	Chang E. and Delzell E.
Report year	2016
Report title	Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers
Document source	Journal of Environmental Science and Health, Part. B. Pesticides, Food Contaminants, and Agricultural Wastes (2016), Vol. 51, No. 6, pp. 402-434
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable

Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The authors conducted a systematic review and meta-analysis of the relationship between glyphosate exposure and risk of lymphohematopoietic cancer (LHC) including non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL), multiple myeloma (MM), and leukaemia. Meta-relative risks (meta-RRs) were positive and marginally statistically significant for the association between any use of glyphosate and risk of NHL (meta-RR = 1.3, 95% confidence interval [CI] 1.0-1.6, based on six studies) and MM (meta-RR = 1.4, 95% CI 1.0-1.9; four studies). Associations were statistically null for HL (meta-RR = 1.1, 95% CI 0.7-1.6; two studies), leukaemia (meta-RR = 1.0, 95% CI 0.6-1.5; three studies), and NHL subtypes except B-cell lymphoma (two studies each). The authors concluded that bias and confounding may account for observed associations and that their meta-analysis was constrained by few studies, a crude exposure metric, and methodologic limitations.

Materials and methods

To identify all potentially relevant articles, the authors searched MEDLINE via PubMed, with additional targeted searches in Web of Science and Google Scholar, along with a review of the bibliographies of recent review articles. Two authors independently reviewed titles and abstracts to agree upon the list of eligible articles. Articles eligible for inclusion in the meta-analysis were original articles describing epidemiological studies that provided numeric point estimates of the RR (i.e., odds ratio, rate ratio, or prevalence ratio) of LHC, including NHL, HL, MM, leukaemia, and any subtypes of these disease entities, associated with individual-level glyphosate exposure, along with corresponding interval estimates (viz., 95% CI) or sufficient raw data to calculate RRs and CIs. Of the 19 eligible articles, 12 pertained to NHL or its subtypes, 2 pertained to HL, 6 pertained to MM, and 3 pertained to leukaemia.

From each eligible study, the authors extracted first author, publication year, study location, study design, study years, source population, number of subjects, proportion of proxy respondents, exposure assessment method, outcome assessment method, confounders adjusted, number of subjects in each exposure category, and RR estimates with CIs. The RR estimates incorporated into the analyses were the most fully adjusted RRs, defined as the RR estimate that took into consideration, by restriction or statistical adjustment, the most covariates that appeared to be confounders.

The authors also qualitatively evaluated the methodological quality of each study in terms of its potential for selection bias, information bias/exposure misclassification, confounding, reporting bias, and other issues affecting validity. Potential for bias was evaluated based on study participant identification strategy, participation rates, investigator blinding, assessment methods for exposures, outcomes, and potential confounders, statistical approach, reporting of results, and other considerations.

The statistical analysis involved both fixed-effects and random-effects estimation of meta-RRs with 95% CIs. Comparison of meta-RR estimates from fixed-effects and random-effects models was one approach to the evaluation of the impact of between-study heterogeneity. As a quantitative measure of between-study heterogeneity, the authors calculated I^2, which represents the percentage of between-study variance in RRs that is attributable to study heterogeneity (as opposed to chance). They also tested for statistically significant between-study heterogeneity based on Cochran's Q statistic, although they acknowledged that this statistical test has low power to detect heterogeneity when there is a limited number of studies.

Results

NHL

The combined meta-RR for NHL overall in association with any use of glyphosate, based on six studies was 1.3 (95% CI 1.0–1.6). The results were identical for random-effects and fixed-effects models, little heterogeneity was indicated by the I^2 value of 0% and the highly non-significant P-value of 0.84 for Cochran's Q. The authors tested for publication bias using Egger's linear regression approach to evaluating funnel plot asymmetry and found no significant asymmetry (one-tailed P-value = 0.20). Using Duval and Tweedie's trim-and-fill approach to adjust for publication bias, the imputed meta-RR for both the random-effects and fixed-effects models was 1.2 (95% CI 1.0-1.6). The authors conducted a number of sensitivity analyses, but the meta-RR was not changed appreciably.

NHL subtypes
The meta-RR for the association between any use of glyphosate and risk of B-cell lymphoma, based on two studies, was 2.0 (95% CI 1.1-3.6) for both random-effects and the fixed-effects models (I^2 = 0%, $P_{heterogeneity}$ = 0.58). The meta-RR for the association between any use of glyphosate and risk of diffuse large B-cell lymphoma, based on two studies, was 1.1 (95% CI 0.5-2.3) for random-effects and the fixed-effects models (I^2 = 0%, $P_{heterogeneity}$ = 0.79). Based on the same two studies, the meta-RR for the association between any use of glyphosate and risk of chronic lymphocytic leukaemia/small lymphocytic lymphoma was 1.3 (95% CI D 0.2-10.0) for the random-effects model and 1.9 (95% CI D 0.9-4.0) according to the fixed-effects model, with significant heterogeneity between the estimates from the 2 studies (I^2 = 84%, $P_{heterogeneity}$ = 0.01). Results for follicular lymphoma from these two studies, by contrast, were not significantly heterogeneous (I^2 = 0%, $P_{heterogeneity}$ = 0.73) with a meta-RR of 1.7 (95% CI 0.7-3.9) for both the random-effects and the fixed-effects models. Lastly, the two studies that reported associations between any glyphosate use and risk of hairy-cell leukaemia yielded a meta-RR of 2.5 (95% CI 0.9-7.3) in the random-effects and fixed-effects models (I^2 = 0%, $P_{heterogeneity}$ = 0.63).

HL
Based on two studies, the meta-RR for any glyphosate use and Hodgkin's lymphoma was 1.1 (95% CI 0.7–1.6) in both the random-effects and the fixed-effects models, with I^2 = 0% and $P_{heterogeneity}$ = 0.36.

MM
The meta-RR for any glyphosate use and risk of MM, based on four studies, was 1.4 (95% CI 1.0–1.9) according to both random-effects and fixed-effects models. Between study heterogeneity was not evident - I^2 = 0% and Cochran's Q statistic P-value was 0.63. Egger's linear regression approach yielded no significant evidence of publication bias (one-tailed P-value for asymmetry = 0.10), while the imputed meta-RR using the trim-and-fill procedure to adjust for publication bias was 1.3 (95% CI 0.9–1.8). Several sensitivity analyses were conducted, but there was minimal effect on the estimated meta-RR for MM.

Leukaemia
The meta-RR based on three studies was 1.0 (95% CI D 0.6–1.5) for both the random-effects model and the fixed-effects estimates (I^2 = 0%, $P_{heterogeneity}$ = 0.92). Publication bias was not assessed because only three studies of leukaemia were available.

Exposure response trends
The authors reviewed the results from the few studies that went beyond an ever exposure metric to look for evidence of an exposure response trend. There was no uniformity in how the studies addressed exposure quantitatively, so no meta-RR could be calculated.

Conclusion

The authors concluded that they found marginally significant positive meta-RRs for any glyphosate use and risk of NHL and MM, and statistically null associations with HL and leukaemia. A statistically significant positive meta-RR for B-cell lymphoma, but not other NHL subtypes, was based on only two studies. Combining these results with recognition of the methodological weaknesses of the small number of existing studies and an overall body of literature that is not strong, consistent, temporally unambiguous, or indicative of a positive biological gradient, the authors concluded that no causal relationship has been established between glyphosate exposure and risk of NHL, HL, MM, leukaemia, or any subtype of LHC.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: The glyphosate meta-RRs took the results from the available studies at face value. The authors had no way to correct for recall bias, confounding, etc. Therefore, the meta-RRs are in error to the extent that the studies included in the meta-analysis are also in error. Chang and Delzell (2016) are clear on this point in their meta-analysis article. Accordingly, glyphosate p-values and confidence intervals for the meta-RRs cannot be taken at face value because they incorporate systematic error or bias. Thus, the argument about the statistical significance/insignificance of the meta-RR for glyphosate is negated. One cannot calculate a valid p-value when there is an uncontrolled systematic error (Greenland S. Randomization, statistics, and causal inference. Epidemiology 1990; 1:421-429).

Further points for clarification:
The results of this meta-analysis have limited relevance for the ongoing evaluation for glyphosate for several reasons. First, the validity of the results from any meta-analysis depends on the validity of the underlying studies. That was the main point in the conclusions reached by Chang and Delzell that the data do not establish a causal relationship between glyphosate and lymphopoietic cancers. Since the publication of the Chang and Delzell meta-analysis, Crump (2020) has illustrated appreciable bias in three of the case-control studies included in this and other glyphosate meta-analyses. Specifically, for the studies of McDuffie et al. (2001), Hardell et al. (2002), and Eriksson et al. (2008), Crump showed that odds ratios (ORs) greater than the null value of 1.0 were reported for 90% or more of the ORs calculated for non-glyphosate pesticides. Therefore, Crump concluded that the findings from these studies were skewed positive, likely due to recall and selection biases, and were not reliable for glyphosate or the other pesticides included in those studies. Accordingly, a meta-RR that includes these studies will be biased and p-values and 95% CIs cannot be taken at face value (Greenland 1990).

A second consideration is that meta-analyses can become out of date as additional results become available. Since the publication by Chang and Delzell, a substantial glyphosate update was published by investigators from the Agricultural Health Study (AHS) (Andreotti et al. 2018). Incorporating these results into a meta-analysis gives a NHL mRR of 1.1 (95% CI 0.9, 1.5) (Table 1).[10]

Table 1. Updated glyphosate NHL meta-analysis calculation

Author	Year	RR	95% CI	weight
McDuffie et al.	2001	1.20	0.83-1.74	23.19%
Hardell et al.	2002	1.85	0.55-6.20	4.57%
DeRoos et al.	2003	1.6	0.9-2.8	14.79%
Eriksson et al.	2008	1.51	0.77-2.94	11.85%
Orsi et al.	2009	1.0	0.5-2.2	10.25%
Andreotti et al.	2018	0.85	0.73-1.00	35.36%
Meta RR		1.1	0.9-1.5	

Lastly, if one wants the best evidence about glyphosate's possible association with NHL and other LHCs, it makes more sense to look at the results for the most frequently exposed population(s), not a weighted average of results based on ever using glyphosate (for as little as one day during a lifetime). Updated results for the most frequently glyphosate exposed population studied have been published from the AHS by Andreotti et al. (see Tables 2 and 3 below for NHL and MM, respectively, based on the supplemental tables from Andreotti *et al*. 2018).

Table 2. NHL results by days of glyphosate use from the AHS

Exposure	# cases	RR	95% CI
No glyphosate	135	1.0	Reference
1 to 14 days	103	0.8	0.6-1.0
14 to 39 days	117	0.9	0.7-1.1
39 to 108 days	107	0.9	0.6-1.1
≥109 days	116	0.8	0.6-1.1

Table 3. Multiple myeloma results by days of glyphosate use from the AHS

Exposure	# cases	RR	95% CI
No glyphosate	30	1.0	Reference
1 to 14 days	23	0.9	0.5-1.6
14 to 39 days	22	0.8	0.4-1.5
39 to 108 days	22	0.9	0.5-1.7
≥ 109 days	22	0.8	0.4-1.5

[10] Random-effects meta-analysis calculations were done using Stata v 14.2 and validated with Episheet (www.krothman.org/episheet xls).

These results for applicators with weeks and months of glyphosate use are more informative than a meta-analysis based on 1 day of glyphosate use or more during a lifetime. It is also noteworthy that the prospective nature of the AHS cohort study precluded case recall bias, of concern in the case-control studies, and that the AHS investigators did a thorough job of controlling for confounding in their RR estimates - in contrast to the sparse control of confounding factors in the case-control studies.

References

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Greenland S. Randomization, statistics, and causal inference. Epidemiology 1990; 1:421-429.

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; 43:1043-1049.

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Assessment and conclusion by RMS:

The study concerns a systematic literature review and meta-analysis on glyphosate exposure and risk of lymphohematopoietic cancers. The work was supported by Monsanto Company. Based on the summary provided above, the RMS is not able to assess the reliability of the publication as the summary is too concise and a detailed analysis including a relevance and reliability assessment is missing. For instance, no details are given on which studies/databases the meta-analysis for NHL overall, NHL subtypes, HL, MM and leukaemia is based, no tabular or graphical results are provided, no results on exposure-response trends are given and the evaluation on bias is not discussed in the summary. Although the RMS acknowledges some of the shortcomings of the meta-analysis as described by the applicant (presented in the commenting box above), based on the information provided the RMS cannot conclude on the reliability of this open-literature study.

A data gap was identified for providing a full assessment of the study including a relevance and reliability assessment (refer to Volume 1, section 2.6.5.1.2 Epidemiological studies). In addition, this study should be discussed in the overall weight-of-evidence approach for carcinogenicity.

B.6.5.18.30. *Public literature – Pesticide use and risk of NHL malignancies in agricultural cohorts from France, Norway and USA (study for which the RMS requested a summary in order to justify the categorization)*

1. Information on the study

Data point:	CA 5.9.4
Report author	Leon M. E. *et al*.
Report year	2019
Report title	Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH consortium
Document source	International Journal of Epidemiology (2019), Vol. 1, No. 48, pp. 1519-1535

Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 -relevance category B)

2. Full summary of the study according to OECD format

In a pooled analysis of three large agricultural cohorts, Leon *et al*. investigated relationships between ever use of 14 selected pesticide chemical groups and 33 individual active chemical ingredients with non-Hodgkin lymphoid malignancies (NHL) overall and for 4 major NHL subtypes. Pesticide use was derived from self-reported history of crops cultivated combined with crop-exposure matrices (France and Norway) or self-reported lifetime use of active ingredients (USA). Cox regression models were used to estimate cohort-specific hazard ratios (HRs) and 95% confidence intervals (CIs), which were combined using random-effects meta-analysis to calculate meta-HRs (mHR). Most mHRs suggested no association between exposures and NHL or its subtypes. Moderately elevated mHRs were seen for: NHL and ever use of terbufos (mHR = 1.18, 95% CI: 1.00-1.39); chronic lymphocytic leukaemia/small lymphocytic lymphoma and deltamethrin (mHR = 1.48, 95% CI 1.06-2.07); and diffuse large B-cell lymphoma and glyphosate (mRR = 1.36, 95% CI 1.00-1.85); as well as inverse associations of NHL with the broader groups of organochlorine insecticides (mHR = 0.86, 95% CI 0.74-0.99) and phenoxy herbicides (mHR = 0.81, 95% CI 0.67-0.98), but not with active ingredients within these groups. The authors concluded that associations of pesticides with NHL appear to be subtype- and chemical-specific.

Materials and methods

The authors evaluated pesticide use and risk of non-Hodgkin's lymphoma (NHL) and subtypes based on a combined analysis of data from three agricultural cohorts: the USA's Agricultural Health Study (AHS), France's Agriculture and Cancer cohort (AGRICAN), and Norway's Cancer in the Norwegian Agricultural Population (CNAP). Taken together, these three cohorts include 316,210 participants: 51,167 participants from the AHS, 127,282 participants from AGRICAN, and 137,821 participants from NCAP. The three cohorts included in this analysis had prospective follow-up from their initial enrolment date as follows:

- AHS - first date of enrolment between 1993-1997 and December 31, 2010 (North Carolina participants) or December 31, 2011 (Iowa participants);
- AGRICAN - first date of enrolment between 2005-2007 and December 31, 2009;
- CNAP – date of identification in 5 agricultural or horticultural censuses (1969, 1974, 1979, 1985, 1989) and December 21, 2012.

Pesticide exposure assessment methodologies were fundamentally different for the US AHS cohort than for the European cohorts. In the AHS, the investigators collected self-reports of the use of specific pesticides directly from cohort members prior to follow-up for health outcomes, while for AGRICAN and NCAP exposure assessment was determined using indirect methods. Specifically:

- AGRICAN cohort members were considered exposed to a pesticide if they reported growing a crop, indicated that they used pesticides on that crop, and a pesticide was registered for use on that crop in France. For example, cohort members who farmed grains were attributed exposure to every pesticide registered for grains for each calendar year during their years of agricultural work.
- CNAP cohort members were considered exposed to a pesticide if: they reported having cultivated a given crop, they possessed spraying equipment or spent money on pesticides, and the active ingredient was registered for use on the crop. As in AGRICAN, CNAP cohort members were attributed exposure for all the pesticides registered for specific crops for each calendar year during their working years.

Cox proportional hazards modelling was used separately for each cohort to estimate hazard ratios (HR) for 14 chemical groups and 32 active ingredients with NHL and 4 NHL subtypes: chronic lymphocytic lymphoma/small lymphocytic lymphoma (CLL/SLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and multiple myeloma/plasma cell leukaemia (MM/PCL). All cohort-specific models were adjusted for age, sex, and animal production. The AGRICAN model was also adjusted for retirement status and the number of crops personally treated with pesticides. For the CNAP cohort model, adjustment was also made for specific pesticides. The cohort-specific HRs were combined using a random-effects meta-analysis model[11] to calculate a mHR.

Results

Focusing on glyphosate (see Table 1, adapted from Table 2 in Leon *et al.*), there was no association between ever using glyphosate and overall risk of NHL. Results for the NHL subtypes showed no positive associations for CLL/SLL, FL, and MM. Lastly, there was a positive association between glyphosate use and DLBCL.

Table 1. Glyphosate NHL findings overall and by subtype from Leon *et al*. 2019

	# cases	mHR	95% CI
NHL	1,131	0.95	0.77-1.18
CLL/SLL	252	0.92	0.69-1.24
DLBCL	221	1.36	1.00-1.85
FL	105	0.79	0.52-1.21
MM	240	0.87	0.66-1.15

DLBCL findings for the individual cohorts were: AHS (HR = 1.20, 95% CI 0.72-1.98), CNAP (HR = 1.67, 95% CI 1.05-2.65) and AGRICAN (HR = 1.06, 95% CI 0.51-2.19).

Conclusion

Focusing on glyphosate, the authors concluded that there was no association for NHL overall or for most subtypes, but that there was an association with DLBCL. They noted that the mHR for glyphosate was higher than the HR from the AHS, though mHR heterogeneity tests did not indicate any appreciable heterogeneity. They also noted that in contrast to the most recent publication from the AHS, they did not control for cigarette smoking, alcohol consumption or family history of cancer, while they did control for animal production and different pesticide active ingredients than in the AHS analysis.

The authors provided a thoughtful commentary on the study's strengths and limitations. Foremost among strengths was the exceptionally large number of individuals in the pooled analyses. The authors also noted that there was no potential for recall bias of pesticide exposures.

Foremost among limitations was concern about exposure misclassification, which the investigators judged as likely to be non-differential. They mentioned the very low correlation with self-reported pesticide information when the AGRICOH method was applied to the AHS population (median correlation for specific pesticides r = 0.07). They also mentioned the inadequacy of an ever versus never exposure metric for characterizing cancer risk from pesticide exposure. Lastly, they mentioned concerns about false-positive findings arising from the evaluation of 14 chemical groups and 32 active ingredients with NHL overall and with 4 NHL subtypes.

3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: Due to an error-prone exposure methodology and the attendant inability to control confounding. We also note that the results for the Norwegian cohort conflict with the AHS results where exposure is determined more specifically and where there is no relationship between glyphosate and DLBCL among individuals in the highest exposed quartile (≥108 days). This publication is considered unreliable.

Further points for clarification:

[11] The random effects model produces a weighted average of findings considering the precision of the cohort-specific findings and the variation of the results among the cohorts.

The glyphosate findings from Leon *et al*. are considered unreliable, largely due to the extremely error-prone indirect methodology used for exposure classification for the AGRICAN and CNAP cohorts. For these cohorts, the investigators used crops farmed to impute exposure for cohort members and they imputed exposure to every pesticide that was registered for every crop in each calendar year. Logically, such a practice would mistakenly attribute exposure for specific pesticides that were not used by individual cohort members. Exposure misclassification was likely to be very substantial as indicated by the negligible median correlation (r = 0.07) observed when this indirect methodology was compared with the directly self-reported exposure information from the AHS. In fact, it is almost certain that the EU exposure cohorts for many pesticides had more unexposed than exposed individuals due to the marked over-attribution of exposure. As such, this method is not reliable for any specific pesticide. Such an error-prone exposure classification methodology would not be acceptable in other fields of epidemiology (e.g., pharmaceutical, dietary, etc.). In addition, this type of exposure methodology precludes controlling for the confounding effect of other pesticide exposures because one cannot differentiate users from non-users of specific pesticides, especially for those farming the same crops.

The authors note exposure misclassification as a limitation and comment that it is probably non-differential, implying that there would be a null bias. The literature on non-differential exposure misclassification never anticipated a situation where the majority of "exposed" individuals may be misclassified for many if not most pesticides. While on average this misclassification should be non-differential and bias towards the null, that is not necessarily true for each of the approximately 470 analyses rendered for the European cohorts (viz., 14 chemical groups, 33 active ingredients, 5 NHL categories, 2 cohorts – 47 exposures x 5 x 2). As Rothman and Greenland (1998) pointed out, in any given study, random fluctuations can lead to bias away from the null (towards a positive or negative association) even if the classification method satisfies all the conditions for being non-differential (viz. on average).

Another important limitation of the findings is the inability to consider the frequency of exposure. In Table 1 (below), HRs by frequency and intensity of exposure are detailed from the recent AHS publication by Andreotti *et al*. (2018). This recent publication has 2 additional years of follow-up beyond that included in the Leon *et al*. analysis for AHS cohort members.

Table 1. DLBCL results by exposure quartile from the most recent AHS publication

Exposure quartiles	Days of use HR (95% CI)	Exposure intensity HR (95% CI)
Q1	1.00 (0.55-1.82)	1.11 (0.60-2.07)
Q2	1.24 (0.70-2.20)	0.94 (0.49-1.80)
Q3	0.94 (0.50-1.75)	1.13 (0.59-2.17)
Q4	0.97 (0.52-1.81)	0.97 (0.51-1.85)
Meta HR[12]	1.04 (0.77-1.40)	1.04 (0.75-1.43)

Andreotti *et al*. (2018) did not find a relationship between glyphosate and DLBCL overall or with increasing duration of exposure or intensity of exposure. Note also that the HR of 1.04 from Andreotti *et al*. is lower than the AHS HR of 1.20 reported by Leon *et al*. This may be due to a number of factors: Leon *et al*.'s exclusion of ~ 4,700 AHS commercial applicators from their analysis that were included in Andreotti *et al*. publication; the 2 years of additional AHS follow-up by Andreotti *et al*.; or differences in the covariates used in the statistical models in the two publications. Interestingly, the glyphosate-DLBCL HR of 1.04 reported by Andreotti *et al*. is very similar to the HR for the AGRICAN cohort (HR = 1.06, 95% CI 0.51-2.19). Notably, the AGRICAN cohort has a very short and recent follow-up period (date of enrolment between 2005-2007 and December 31, 2009), so the extent of the exposure misclassification would be much less than for the CNAP cohort. It is clear that the glyphosate-DLBCL association in Leon *et al*. is largely a result of the findings for the Norwegian cohort (HR = 1.67, 95% CI 1.05-2.65).

In conclusion, the glyphosate-DLBCL association reported by Leon *et al*. is deemed unreliable due to an error-prone approach to exposure assessment for 2 of the 3 cohorts. The lack of any relationship between glyphosate and DLBCL with increasing duration and intensity of exposure in the most recent AHS publication (Andreotti *et al*. 2018), with a much more accurate exposure assessment, would also argue against a causal interpretation for glyphosate and DLBCL.

[12] All meta-analysis calculations in this section are random effects calculations done with Stata version 14 and verified with Episheet. Fixed effects calculations produced the same estimates.

References

Andreotti G, Koutros S, Hofmann JN, *et al*. Glyphosate Use and Cancer Incidence in the Agricultural Health Study. J Natl Cancer Inst. 2018; 110(5): 509-516.

Rothman KJ, Greenland S. 1998. Modern epidemiology. 2nd ed. Philadelphia (PA): Lippincott Williams & Wilkins.

Assessment and conclusion by RMS:

In a pooled analysis of three large agricultural cohorts, the authors investigated relationships between ever use of 14 selected pesticide chemical groups and 33 individual active chemical ingredients with non-Hodgkin lymphoid malignancies (NHL) overall and for 4 major NHL subtypes. Based on the summary provided above, the RMS is not able to assess the reliability of the publication as the summary is too concise and a detailed analysis including a relevance and reliability assessment is missing. In addition, based on the summary alone the 'further points of clarification provided by the applicant' could not be followed. Although the RMS acknowledges some of the shortcomings of the meta-analysis as described by the applicant (presented in the commenting box above), based on the information provided by the applicant the RMS cannot make a final conclusion on the reliability of this open-literature study. However, it does seem that this study could be reliable based on:

(i) the sample size is unprecedented with more than 300 000 individuals covered;

(ii) the study is a cohort which has much more weight than case-control studies i.e., very limited of null selection bias, exposure a genuinely characterized, the covered period of time is long enough;

(iii) in this cohort participants are enrolled via national systems of social security or public health which allows an adequate tracing of the participants and their exposure throughout the cohort, and collected information are more reliable

(iv) the methodology chosen (Cox model) seems adequate

Therefore, a data gap was identified for providing a full assessment of the study including a relevance and reliability assessment (refer to Volume 1, section 2.6.5.1.2 Epidemiological studies). In addition, this study should be discussed in the overall weight-of-evidence approach for carcinogenicity.

B.6.5.18.31. *Public literature – Exposure to glyphosate based herbicides and risk for NHL: a meta-analysis and supporting evidence (study for which the RMS requested a summary in order to justify the categorization)*

1. Information on the study

Data point:	CA 5.9.4
Report author	Zhang L. *et al.*
Report year	2019
Report title	Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: a meta-analysis and supporting evidence
Document source	Mutation Research, Reviews in Mutation Research (2019), Vol. 781, pp. 186-206
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability: as provided in the AIR5 dossier (KCA 9)	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)

2. Full summary of the study according to OECD format

The authors conducted a glyphosate/non-Hodgkin's lymphoma (NHL) meta-analysis that includes the 2018 update of the Agricultural Health Study (AHS) cohort along with five case-control studies. Using the highest exposure groups when available in each study, they reported that the overall meta-relative risk (meta-RR) of NHL in glyphosate exposed individuals was RR = 1.41 (95% confidence interval [CI] 1.13-1.75). They also performed a secondary meta-analysis using high-exposure groups with the 2005 AHS results and calculated a meta-RR for NHL of 1.45 (95% CI 1.11-1.91). Multiple sensitivity analyses did not change results appreciably. After reviewing the toxicological findings for glyphosate and finding them to provide evidence for an increased cancer risk, the authors concluded that the epidemiological studies suggest a compelling link between glyphosate exposure and increased risk for NHL.

Materials and methods

The literature search was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis using PubMed in November 2017, updated in March 2018, and again in August 2018. Searches included all cohort, case-control, and cross-sectional studies. No language restrictions were applied. From the PubMed search, 857 studies were identified. Additionally, 52 studies were identified from the IARC evaluation of the carcinogenicity of glyphosate, the U.S. EPA review of glyphosate, and the WHO JMPR report on glyphosate, for a total of 909 studies. After 43 duplicates were excluded, 866 studies were initially screened by title and abstract, of which 850 were excluded because they were reports, correspondence, reviews, irrelevant studies (animal, mechanistic, para-occupational), or did not include the exposure or outcome of interest. When the final 16 epidemiologic studies were identified, 10 studies were further excluded because (1) they did not report RRs, ORs, or the data needed to calculate either, (2) the cohort overlapped with another study, or (3) they did not specify whether the lymphomas were specifically NHL. For studies including overlapping cohorts, the authors used results from the most complete and updated analysis with the greatest number of participants.

The methodological quality of the cohort and case-control studies included in the meta-analyses was assessed independently by two co-authors using the Newcastle Ottawa Scale. Studies were evaluated based on selection, comparability, and outcome or exposure (in nine categories). Cohort studies were evaluated based on (1) representativeness of the cohort, (2) selection of non-exposed, (3) ascertainment of exposure, (4) demonstration that the outcome of interest was not present at the start of study, (5) comparability of the cohort on the basis of controlling for other pesticide use, (6) age, (7) assessment of NHL outcome, (8) sufficiency of follow-up length, and (9) response rate. Case-control studies were evaluated on (1) the validation of cases, (2) representativeness of cases, (3) selection of controls, (4) absence of disease in the controls, (5) whether the study controlled for other pesticide use and (6) age, (7) exposure assessment, (8) concordance of method among cases and controls, and (9) similarity of response rate among both groups. Each study was awarded a maximum of one point for every item that was satisfied, with a total of 9 available points. According to the quality assessment, the highest quality study in either design category was the AHS 2018 cohort (Andreotti *et al*.). The highest quality case-control study was Eriksson *et al*. (2008), while the lowest quality studies were case-control studies by McDuffie *et al*. (2001) and Orsi *et al*. (2009).

The authors calculated meta-RRs and 95% confidence intervals (CI) using both fixed-effects and random-effects models. They favoured the fixed effects results in their interpretation because they considered random effects estimates to be more conservative. They assessed between-study heterogeneity using the summary-variance method. Lastly, they evaluated publication bias through funnel plots, Egger's test, and Begg's test.

Results

The authors reported a fixed-effects glyphosate-NHL meta-RR of 1.41 (95% CI 1.13-1.75) based on 6 studies, including the 2018 AHS update (Andreotti *et al*., 2018). The corresponding random-effects meta-RR was 1.56 (95% CI 1.12-2.16). Replacing the 2018 AHS findings with the 2005 AHS results (De Roos *et al*., 2005), used in the two previously published meta-analyses, resulted in a meta-RR of 1.45 (95% CI 1.11-1.91). The results were similar for the random-effects model. There was little evidence of heterogeneity or publication bias in the funnel plots, Egger's test ($p = 0.185$), or Begg's test ($p = 0.851$).

The authors also conducted analyses using the longest exposure duration results, which yielded a meta-RR of 1.41 (95% CI 1.13-1.74). When the AHS 2005 findings were substituted in this analysis for the AHS 2018

findings, the meta-RR was 1.56 (95% CI 1.17-2.06). When evaluating three studies with only the highest levels of exposure, the meta-RR was 1.36 (95% CI 1.06-1.75).

Conclusion

The authors concluded that their analyses demonstrate a statistically significant increased NHL risk in glyphosate exposed individuals (meta-RR = 1.41, 95% CI 1.13-1.75), consistent with the two previous meta-analyses. They also concluded, based on a review of the toxicology data for glyphosate, that the association identified in the epidemiologic studies was supported by toxicological data for glyphosate, as opposed to an added ingredient in glyphosate formulations. Overall, they concluded that the evidence from human, animal, and mechanistic studies supports a compelling link between exposures to glyphosate and increased risk for NHL.

References

Andreotti G, Koutros S, Hofmann JN, *et al*. Glyphosate use and cancer incidence in the Agricultural Health Study. J Natl Cancer Inst 2018; 110(5): 509-516.

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3. Assessment and conclusion

Assessment and conclusion by applicant:

5.4.1 case b) Relevant but supplementary information: Meta-analyses cannot overcome the limitations of the studies included. This publication is considered unreliable.

Further points for clarification:
The meta-RRs from the Zhang *et al*. (2019) publication are considered to be unreliable for the ongoing glyphosate evaluation for similar reasons noted for the previous two glyphosate meta-analyses (Schinasi and Leon, 2014; Chang and Delzell, 2016) - taking a weighted average of results from the existing glyphosate NHL studies cannot correct for the uncorrected biases from the underlying studies. Paramount among these uncorrected biases are recall and selection biases in the case-control studies and failure to control for confounding factors comprehensively in many of the case-control analyses (with the possible exception of De Roos *et al*. 2003). Notably, Crump (2020) illustrated appreciable bias in three of the case-control studies included in this and other glyphosate meta-analyses. Specifically, for the studies of McDuffie *et al*. (2001), Hardell *et al*. (2002), and Eriksson *et al*. (2008), Crump showed that odds ratios (ORs) were greater than the null value of 1.0 for 90% or more of the ORs calculated for non-glyphosate pesticides. Therefore, Crump concluded that the findings from these studies were skewed positively, likely due to recall and selection biases, and were not reliable for glyphosate or for the other pesticides included in those studies. Accordingly, an mRR that includes these studies will be biased and p-values and 95% CIs are not valid (Greenland, 1990).

There are also considerations that relate to how Zhang *et al*. selected data points for their analysis. The authors assert that their analysis is an advancement over previous meta-analyses because they targeted biologically relevant findings (highest cumulative exposure or longest lag categories) from the studies that were included in previous meta-analyses and include the updated findings from the AHS cohort (Andreotti *et al*, 2018). When no high exposure or long latency findings were available, they defaulted to findings for any glyphosate exposure. Their primary analysis yielded an mRR of 1.41 (95% CI 1.13-1.75) and they concluded that their results support a relationship between glyphosate and NHL. The meta-RR by Chang and Delzell (2016) and the corrected meta-RR by Schinasi and Leon (2014, corrected in IARC 2015) were 1.3 (95% CI 1.0-1.6).

A closer look at the individual data points for the studies included in this analysis by Zhang *et al*. (2019) suggests that the data points selected were often not controlled for confounding factors, in contrast to the other meta-analyses that selected the most adjusted finding from the individual studies (Schinasi and Leon, 2014 corrected in IARC 2015; Chang and Delzell, 2016) and, in some instances, seems to have been chosen arbitrarily. Specifically:

- For McDuffie *et al*. (2001), Zhang *et al*. selected the OR for greater than 2 days per year of reported use (OR 2.12, 95% CI 1.2-3.7) that was adjusted only for age and province. Previous meta-analyses had used the OR for "ever use" (OR 1.2, 95% CI 0.83-1.74) that was adjusted for significant medical variables (history of measles, mumps, cancer, allergy desensitization shots, and family history of cancer in a first-degree relative), age and province. Neither OR (viz., 2.12 or 1.20) was adjusted for other pesticides or other farming exposures.

- For Hardell *et al*. (2002), the authors used the ever exposed OR of 1.85 (95% CI 0.55-6.20) that was used in previous meta-analyses.

- For DeRoos *et al*. (2003), the authors used the first stage OR estimate for any glyphosate exposure of 2.10 (95% CI 1.10-4.00). Previous meta-analyses used the second stage (hierarchical regression) OR of 1.6 (95% CI 0.9-2.8). This choice by Zhang *et al*. had nothing to do with picking a higher exposure or longer lag period. There is no rationale consistent with their stated analysis approach that justifies picking the first stage (higher) OR for glyphosate.

- For Eriksson *et al*. (2008), the authors picked the OR for greater than 10 days of glyphosate use in a lifetime (OR 2.36, 95% CI 1.04-5.37). Other meta-analyses have used the any glyphosate exposure OR of 1.51 (95% CI 0.77-2.94) that was adjusted for other pesticides (IARC 2015/Schinasi and Leon [2014] corrected; Chang and Delzell, 2016). In Eriksson's paper, the any glyphosate exposure OR unadjusted for other pesticides was 2.02 (95% CI 1.10-3.71) versus 1.51 (95% CI 0.77-2.94) adjusted, so it is clear that the value used by Zhang *et al*. is confounded.

- For Orsi *et al*. (2009), the authors used the ever exposed OR of 1.0 (95% CI 0.5-2.2) that was used by other meta-analysis authors.

- For Andreotti *et al*. (2018), the authors chose the HR of 1.12 (95% CI 0.83-1.51) corresponding to the highest days of use quartile with the longest lag period of 20 years. This value came from the supplemental tables for the Andreotti *et al*. paper. There was any number of values that could have been chosen from this study. For example, the NHL RR for the highest exposure intensity quartile with a 15-year lag was RR 0.94 (95% CI 0.71-1.24). Arguably, there are other values from Andreotti *et al*. that could have been chosen. All would be lower than the value selected for the analysis.

Considering this most recent meta-analysis in the context of the previous meta-analyses, the data points selected were typically not adjusted for other exposures, which resulted in including higher OR values than were included in previous meta-analyses and a marginally higher meta-RR for glyphosate and NHL. Nothing was done in this meta-analysis to address the uncontrolled biases in the underlying studies. If one wants the best evidence about glyphosate's possible association with NHL, it makes more sense to look at the results for the most frequently exposed population(s) from a prospective cohort study with no potential for case recall bias and a comprehensive analysis of confounding factors (Table 1 from Andreotti *et al*. 2018, supplemental tables).

Table 1. NHL results by days of glyphosate use from the AHS

Exposure	# cases	RR	95% CI
No glyphosate	135	1.0	Reference
1 to 14 days	103	0.8	0.6-1.0
14 to 39 days	117	0.9	0.7-1.1
39 to 108 days	107	0.9	0.6-1.1
≥109 days	116	0.8	0.6-1.1

These data provide no indication of an association between frequent glyphosate exposure and NHL.

References

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Schinasi L, Leon ME. 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 2014; 11: 4449-4527.

Assessment and conclusion by RMS:

The authors conducted a meta-analysis on studies investigating the relation between non-Hogdkin’s lymphoma (NHL) including the 2018 update of the Agricultural Health Study (AHS) cohort along with five case control studies. Based on the summary provided above, the RMS is not able to assess the reliability of the publication as the summary is too concise and a detailed analysis including a relevance and reliability assessment is missing. In addition, based on the summary alone the ‘further points of clarification provided by the applicant’ could not be followed. Although the RMS acknowledges some of the shortcomings of the meta-analysis as described by the applicant (presented in the commenting box above), based on the information provided the RMS cannot conclude on the reliability of this open-literature study.

A data gap was identified for providing a full assessment of the study including a relevance and reliability assessment (refer to Volume 1, section 2.6.5.1.2 Epidemiological studies). In addition, this study should be discussed in the overall weight-of-evidence approach for carcinogenicity.

Appendix to B.6.5 Overview of publications related to long-term toxicity and carcinogenicity that are classified by the applicant as "non-relevant after detailed assessment of full-text article"

To complement the standard toxicity studies, the applicant has performed a literature search in accordance with the EFSA Guidance document EFSA Journal 2011;9(2):2092 "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009". The results were categorized as "non-relevant", as "potentially relevant" or to be of "unclear relevance" following a rapid assessment. For the two latter categories, the full-text documents were reviewed in detail and then categorized as "non-relevant" or "relevant". The articles considered relevant were categorized as A (providing data for establishing or refining risk assessment parameters), as B (articles relevant to the data requirement but in opinion of the applicant only to provide supplementary information that does not alter existing risk assessment) or as C (articles of unclear relevance).

More details on the literature search, including tables describing the studies in categories A, B and C can be found in Volume 3 CA section B.6.10.1.

For one study related to carcinogenicity that was classified by the applicant as "non-relevant after detailed assessment of full-text articles", the AGG requested study summaries to further justify the categorization of the information. The justification provided by the applicant was reviewed by the RMS and the assessments are presented in this appendix for this study.

Overview of study categorised by the applicant as non-relevant[13] after rapid assessment of title/abstract for which the RMS requested a summary in order to further justify the categorization.

No.	Technical section	Author	Year	Title
1357	Toxicology and metabolism	George J. et al.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach.

Data point	CA 9
Author	George J. *et al.*
Year	2010
Title	Studies on glyphosate induced carcinogenicity in mouse skin: a proteomic approach.
Document source	Journal of Proteomics (2010), Vol. 73, No. 5, pp. 951-64
Short description of literature article	The present investigation was carried out to study the carcinogenic potential of glyphosate and to identify differentially expressed proteins, using 2-dimensional gel electrophoresis and mass spectrometry analysis after treatment with glyphosate, a known tumour promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) and tumour initiator, 7, 12-dimethylbenz[a]anthracene (DMBA) in mouse skin. As test item commercial formulation of the herbicide glyphosate Roundup Original (glyphosate 41%, POEA ≅15%, Monsanto Company, St. Louis, MO, USA) was used, which contains 360 g/L glyphosate acid equivalent as the isopropylamine salt and was procured from a local market. Male Swiss albino mice (12-15 g body weight [bw]) were taken from the Indian Institute of Toxicology Research (IITR) animal breeding colony and acclimatized for 1 week. The ethical approval for the experiment was obtained from the institutional ethical committee. The animals were kept under standard laboratory conditions (temperature 23 ± 2 °C, relative humidity 55 ± 5%) and were fed with synthetic

[13] The applicant has provided an Excelsheet in which all articles are presented that are considered non-relevant after rapid assessment (based on title/abstract). These were checked by the RMS and for the articles listed in the table the RMS has requested a summary in order to further justify the categorization of the study.

	pellet basal diet (Ashirwad, Chandigarh, India) and tap water *ad libitum*. Animals were randomly divided into 8 groups of 20 animals each.
Short description of findings	Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, DMBA and 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. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The upregulation of calcyclin, calgranulin-B and downregulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumour promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.
Justification as provided in the AIR5 dossier (KCA 9)	Not relevant by full text: The test material was Roundup Original (containing POEA) and not the reference formulation MON 52276. As this is not the EU representative formulation, the article is not relevant to the glyphosate EU renewal. Furthermore, in the absence of a concurrent control for each of the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or to one of the other components.
RMS comments	The authors of the study aimed to investigate the carcinogenic potential and to identify differentially expressed proteins in mouse skin upon topical application of a glyphosate formulation. The RMS does agree with the applicant's justification that the study has no relevance to the assessment on glyphosate as the test material was not glyphosate alone or the reference formulation MON 55276. As no individual components were investigated, it is not possible to determine whether any observed effects in this study are related to glyphosate or to one of the other components or the synergistic effect of the components in the formulation.

B.6.6. REPRODUCTIVE TOXICITY

Refer to separate RAR B.6.6.

B.6.7. NEUROTOXICITY

Refer to separate RAR B.6.7-B.6.10.

B.6.8. OTHER TOXICOLOGICAL STUDIES

Refer to separate RAR B.6.7-B.6.10.

B.6.9. MEDICAL DATA AND INFORMATION

Refer to separate RAR B.6.7-B.6.10.

B.6.10. REFERENCES RELIED ON

Refer to separate RAR B.6.7-B.6.10.