European Commission

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and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Glyphosate

Volume 3 – B.6.3 (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. TOXICOLOGY AND METABOLISM DATA

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

Refer to separate RAR B.6.1-B.6.2.

B.6.2. ACUTE TOXICITY

Refer to separate RAR B.6.1-B.6.2.

B.6.3. SHORT-TERM TOXICITY

B.6.3.1. Oral 28-day studies

B.6.3.1.1. Oral 28-day study in rat - study 1

Data point	CA 5.3.1/001
Report author	[redacted]
Report year	1991 (study report)
Report title	28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990)
Report No	[redacted].881.28 DDR
Document No	Not reported
Guidelines followed in study	OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	The following organs were not examined in the gross pathology evaluation: epididymides, peripheral nerve, prostate, skeletal muscle and bone, spinal cord, thymus, vagina. The following organs were not weighed: epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Urinalysis was not performed. Reticulocyte count, platelet count, urea, total cholesterol pa rameters were not measured. Thyroid hormone levels were not determined. Deviations from the current version of OECD 407 (2008) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 407.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Data point	CA 5.3.1/002
Report author	[redacted]
Report year	1st amendment (June 1994); 2nd amendment (November 1994)
Report title	Amendment to Final Report. 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate
Report No	[redacted].881.28 DDR
Document No	Not reported
Guidelines followed in study	Refer to CA 5.3.1/001
Deviations from current test guideline (OECD 407, 2008)	Refer to CA 5.3.1/001
Previous evaluation	Refer to CA 5.3.1/001

GLP/Officially recognised testing facilities	Refer to CA 5.3.1/001

Data point	CA 5.3.1/003
Report author	[redacted]
Report year	1991 (Appendices to study report)
Report title	28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990)
Report No	[redacted].881.28 DDR
Document No	Not reported
Guidelines followed in study	Refer to CA 5.3.1/001
Deviations from current test guideline (OECD 407, 2008)	Refer to CA 5.3.1/001
Previous evaluation	Refer to CA 5.3.1/001
GLP/Officially recognised testing facilities	Refer to CA 5.3.1/001

Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable but with restrictions (reliable with restrictions) as there are indications of lung infections based on the high on the incidence of lung congestion, interstitial pneumonia etc among both control and treated animals. The deviations mentioned are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 407.

Executive Summary

In a 4–week toxicity study, groups of five male and five female Wistar rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm (equivalent to 0, 17.6, 178.5, 1894.9 or 1987.5 mg/kg bw/day in males and 21.6, 223.3, 2250.8 or 2129.7 mg/kg/day in females). A high-dose recovery group was administered 20000 ppm for 28 days and then were followed for 14 days without treatment before being sacrificed.

General clinical observations were done daily. Body weights and food consumption were assessed in weekly intervals. Haematological and blood biochemistry parameters were evaluated during week 4 of dosing. At the end of the scheduled period, the animals were killed and subjected to a post-mortem examination and selected organs were weighed and tissues were taken for subsequent histopathology examination.

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urine incontinence in the mid- and high-dose groups but not in the high-dose recovery group. There were no notable intergroup differences in body weights, food consumption, or haematological parameters. With regards to clinical chemistry parameters, there was a statistically significant increase in blood urea nitrogen (BUN) level, however, without a clear dose-response relationship and a statistically significant increase in the activity of glutamic pyruvic transaminase (SGPT) at the high-dose level. There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

RMS conclusion: in disagreement with the previous assessment and the NOAEL proposed by the applicant, the RMS considers the effects reported at the top dose of 2000 ppm as not adverse. The increased glutamic pyruvic transaminase (SGPT/ALAT) levels in males and females at 20000 ppm were increased by 40-45% compared with controls, which is below the cut-off of 50% as proposed by JMPR as a starting point of adversity. In addition, the increase in blood urea nitrogen (BUN) was not considered treatment-related due to a lack of a clear dose-response relationship.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	Solid, odourless white coloured crystals
Lot/Batch#:	FSG 03090 H/05 March 1990 / Batch No. 60
Purity:	96.8%
Stability of test compound:	Fairly stable for 30 days under ambient temperature and stored in polyethylene lined stainless steel drums

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	8 weeks
Sex:	Male and female
Weight at dosing:	♂ group means 130 – 174 g; ♀ group means 108 – 154 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed, *ad libitum,*
Water:	Deep borewell water passed through activation charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Groups of 5 rats/ sex in steam sterilized standard polypropylene rat cages with stainless steel top grill
Environmental conditions:	Temperature: 23 ± 2°C Humidity: 66 ± 2% Air changes: 10 – 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: November 1990 to January 1991

Animal assignment and treatment:
Groups of five male and five female Wistar rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations of 0 (control), 20, 2000, or 20000 ppm.

Table 6.3.1.1-1: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) [redacted] 1991): Animal assignment

Test group	Dose Level [ppm]	Males	Females
Control	0	5	5
Low	200	5	5
Intermediate	2000	5	5
High	20000	5	5
High Recovery	20000	5	5

The required amount of finely ground test compound was weighed and mixed manually with 0.5 kg powdered rat feed to prepare the premix. The premix was added in portions to the remaining quantity of feed and mixed. To the control group feed, the powdered rat feed was mixed for 20 minutes. Prepared feed was sampled at different levels for assaying the test compound concentration.

All test groups received diet specifically prepared for the group ad libitum. Animals were treated for seven days a week for four weeks. The high dose recovery group received powdered normal rat feed for two weeks following four weeks of treatment.

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
General clinical observations were done twice daily. Cage side observations included changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern.

Body weight
Individual body weights were recorded at the end of each week.

Food consumption and utilisation
Daily feed consumption per cage measured during the last two days of the week.

Ophthalmoscopic examination
Ophthalmoscopic eye examinations were not performed.

Haematology and clinical chemistry
One day prior to sacrifice, blood smears from surviving rats were made by tail clipping and the blood smears were stained by Wright's stain. At the end of the study all the surviving animals were fasted overnight (water allowed) and blood was collected from abdominal aorta under ether anaesthesia.

For haematology, differential leucocyte counts were done manually. Fractions of blood were taken for coagulation time. For haematology and plasma separation blood was heparinized. The following haematological parameters were measured: white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

For clinical chemistry analysis the following parameters were measured: glucose, total bilirubin, creatinine, glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), enzymatic blood urea nitrogen (BUN), albumin, alkaline phosphatase, total protein, sodium, potassium, calcium, and inorganic phosphorus.

Urinalysis
Not performed.

Sacrifice and pathology
At the end of the study, rats were fasted overnight and sacrificed by total blood collection under either anaesthesia. After sacrificed, a detailed gross necropsy was conducted. The following organs were collected, weighed and preserved from every animal: adrenals (both), gonads (both), kidneys (both), and liver. The relative organ weights were determined as a percentage of body weight. Tissue samples from brain, lungs, heart, eye (Bouin's fluid), spleen, lymph nodes, (mesenteric) oesophagus, stomach, small and large intestines, salivary gland, liver, pancreas, kidneys (both), urinary bladder, testes/ovaries (both), seminal vesicles/uterus, pituitary, thyroid and adrenals (both) were preserved in neutral buffered formalin for processing and 5 µm sections were stained. The tissues sections were studied for histopathological changes. All the tissues from all animals in control and high-dose group were subjected to detailed histopathological studies.

Statistics

Body weight and feed consumption were compared using the Bartlett's test for homogeneity of variance and one-way classification analysis of variance (ANOVA) and Dunnet's multiple pairwise comparison. The clinical laboratory analysis data and organ weight data from the control group were compared with the data of treated groups by the Bartlett's test for homogeneity of variance followed by ANOVA, where the variances proved to be heterogeneous, the data was transformed using the appropriate transformation. If ANOVA of homogeneous data was significant, Dunnet's pairwise comparison procedure was used to compare the treated group with the control group individually. All analyses were evaluated at 5% probability level.

II. RESULTS AND DISCUSSION

A. MORTALITY

There was no mortality in any of the study groups during treatment or recovery period.

B. CLINICAL OBSERVATIONS

In general there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urine incontinence in the mid- and high-dose groups but not in the high-dose recovery group.

C. BODY WEIGHT

Body weight gains were comparable between treatment groups and control animals and there were no statistically significant differences. Results are presented in the following table:

Table 6.3.1-2: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) (., 1991): Intergroup comparison of mean body weights (both sexes combined)

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	Combined Sex (5 males + 5 females)				
0	146±8.77	149±10.38	173±16.84	196±27.18	209±32.01
200	**131±12.89*** ↓	138±17.63	159±20.05	175±21.48	186±28.17
2000	144±13.69	153±14.27	172±20.54	195±29.62	207±40.77
20000	149±15.46	150±16.18	174±26.61	189±30.01	207±42.74
20000 (recovery group)	149±15.95	155±14.61	173±26.79	195±32.11	206±40.81

* Significant at P = 0.05 over control group value.
↓ Decreased

Table 6.3.1-3: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) (1991): Intergroup comparison of mean body weights for males

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	Males				
0	150±7.87	156±8.00	186±9.94	220±11.44	238±14.24
200	**139±10.06*** ↓	150±11.87	172±15.56	194±9.49	210±12.92
2000	154±8.99	164±11.08	189±10.26	220±15.65	242±23.87
20000	161±9.65	163±10.06	198±9.49	216±11.17	245±17.81
20000 (recovery group)	162±9.84	165±10.06	195±13.16	221±16.41	240±18.51

* Significant at P = 0.05 over control group value.
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased

Table 6.3.1-4: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Intergroup comparison of mean body weights for females

Time point	Weekly body weight [g]				
	0	1	2	3	4
Dose level [ppm]	Females				
0	142 ± 8.17	142 ± 7.92	159 ± 7.95	172 ± 9.53	181 ± 10.73
200	**122 ± 9.38*** ↓	127 ± 15.66	145 ± 14.60	157 ± 9.12	162 ± 13.37
2000	134 ± 9.53	143 ± 8.67	154 ± 10.24	169 ± 8.07	172 ± 8.53
20000	138 ± 10.95	137 ± 8.07	150 ± 9.32	162 ± 7.21	168 ± 10.33
20000 (recovery group)	136 ± 7.27	145 ± 11.37	151 ± 14.46	168 ± 18.30	172 ± 22.69

* Significant at P = 0.05 over control group value.
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

All groups receiving Glyphosate performed similarly to their respective controls. Test compound intakes are presented in the following table:

Table 6.3.1-5: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Feed intake for combined sexes

Time point	Feed intake mean g/rat/day [g]				
	0	1	2	3	4
Dose level [ppm]	Combined Sex (5 males + 5 females)				
0	17±2.83	18±0.71	19±1.41	16±1.41	16±1.41
200	16±1.41	16±0.71	15±2.12	16±1.41	15±0.07
2000	16±2.83	16±1.41	19±2.12	16±1.41	19±2.12
20000	13±3.54	17±1.41	18±1.41	15±2.83	22±2.12
20000 (recovery group)	15±2.83	17±4.95	18±2.83	17±0.71	20±4.24

Table 6.3.1-6: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Feed intake for males

Time point	Feed intake mean g/rat/day [g]				
	0	1	2	3	4
Dose level [ppm]	Males				
0	19	18	20	17	17
200	17	15	16	17	15
2000	18	17	20	17	17
20000	15	18	19	17	23
20000 (recovery group)	17	20	21	17	22

Table 6.3.1-7: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Feed intake for females

Time point	Feed intake mean g/rat/day [g]				
	0	1	2	3	4
Dose level [ppm]	Females				
0	15	17	18	15	15
200	15	16	13	15	15
2000	14	15	17	15	20
20000	10	16	17	13	20
20000	13	13	17	16	18

Table 6.3.1-8: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Test compound intake

	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (re-covery)	0	200	2000	20000	20000 (re-covery)
Dose [mg/kg bw/day]	0	17.6	178.5	1894.9	1987.5	0	21.6	223.3	2250.8	2129.7

E. OPHTHALMOSCOPIC EXAMINATION

Specific eye examinations were not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group for most of the parameters examined during the haematology evaluation. Haemoglobin values, however, showed a decrease in the high-dose recovery group when compared with the high-dose groups for males and females.

Table 6.3.1-9: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Intergroup comparison of selected haematology parameters (mean ± SD)

Parameter	Combined Sex (5 males + 5 females)				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
Hb [g/dL]	16.61±1.80	16.64±1.12	18.46±2.43	16.36±1.98	14.95±0.40↓

↓ Decreased, compared to the high-dose group

Table 6.3.1-10: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Selected haematology parameters (mean ± SD) for males

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
Hb [g/dL]	17.12±2.06	16.70±1.40	17.76±1.34	20.44±1.73	14.80±0.34↓

↓ Decreased, compared to the high-dose group

Table 6.3.1-11: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Selected haematology parameters (mean ± SD) for females

Parameter	Females				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
Hb [g/dL]	16.10±1.54	16.58±0.92	14.96±1.44	16.48±0.74	15.10±0.43↓

↓ Decreased, compared to the high-dose group

Blood clinical chemistry

There were no treatment-related differences noted in any dose group for most of the parameters examined during the clinical chemistry evaluation. There was a significant increase in SGPT in both sexes at the high-dose level. These changes were not evident in the 14 day recovery group. A significant increase in BUN at all dose levels in females and a significant decrease in calcium in females at the low-dose level were observed. Lastly there was an apparent increase in calcium in the high-dose recovery group, but in the absence of a concurrent control sample the significance of this is in doubt.

Table 6.3.1-12: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Intergroup comparison of selected clinical chemistry parameters (mean ± SD)

Parameter	Combined Sex (5 males + 5 females)				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
SGPT [IU/L]	32.80±6.76	37.00±7.07	30.20±5.35	**46.50±8.24*↑ (+40%)**	23.90±5.41↓
BUN [mg/dL]	23.60±2.68	**28.10±3.48*↑**	24.50±2.84	**28.10±2.38*↑**	24.20±3.12
Calcium [mg/dL]	9.35±0.14	**9.20±0.08*↓**	9.29±0.12	9.25±0.11	10.00±0.58↑

* Significant at P = 0.05 over control group value

↓ Decreased; ↑ Increased

Table 6.3.1-13: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Selected clinical chemistry parameters (mean ± SD) for males

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
SGPT [IU/L]	36.2±7.29	39.4±8.53 ↑	27.2±5.89 ↓	**50.4±6.88*** ↑ **(+39%)**	27.0±6.40 ↓
BUN [mg/dL]	24.6±3.51	26.0±3.08 ↑	23.8±1.19	26.2±1.10 ↑	25.2±3.96 ↑
Calcium [mg/dL]	9.26±0.15	9.18±0.08	9.26±0.15	9.18±0.08	10.00±0.74 ↑

* Significant at P = 0.05 over control group value
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased; ↑ Increased

Table 6.3.1-14: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Selected clinical chemistry parameters (mean ± SD) for females

Parameter	Females				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
SGPT [IU/L]	29.4±4.56	34.6±5.03 ↑	33.2±2.68 ↑	**42.6±8.20*** ↑ **(+45%)**	20.8±0.84
BUN [mg/dL]	22.6±1.14	**30.2±2.59*** ↑	**25.2±3.63*** ↑	**30.0±1.58*** ↑	23.2±1.92
Calcium [mg/dL]	9.44±0.05	**9.22±0.08*** ↓	9.32±0.08	9.32±0.08	10.00±0.46 ↑

* Significant at P = 0.05 over control group value
Re-assessment performed using a statistical program. Based on the data set, the most appropriate t-test procedure was selected by the program.
↓ Decreased; ↑ Increased

G. URINALYSIS
Not performed.

H. NECROPSY

Organ weights
There were no intergroup differences in either sex.

Gross pathology
At the high dose, there was an increase incidence of petechiae and ecchymosis and two incidences of focal congestion in the lungs. In the recovery group, there was one occurrence of enteritis and thickening of splenic capsule and two incidences of petechiae in the lungs. These changes appear to be incidental.

Table 6.3.1-15: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Summary incidence of gross pathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (re-covery group)	0	200	2000	20000	20000 (re-covery group)
Pancreatic lymph node - enlarged	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Lungs – Petechiae	0/5	0/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5
Lungs – Ecchymosis	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Lungs – Focal congestion	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
Small intestine – Enteritis	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Spleen Constriction – Thickening of capsule	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5

Histopathology
There were no treatment-related findings reported during the histopathological evaluation. Lymphoid hyperplasia in the colon and lungs were more frequently observed in the high-dose group; however, this finding was not considered treatment related because the severity remained unaltered as compared to the control animals.

Table 6.3.1-16: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted], 1991): Summary incidence of histopathological findings

Findings	Dose level [ppm]			
	Males		Females	
	0	20000	0	20000
Lungs				
Congestion	4/5	4/5	5/5	5/5
Lymphoid hyperplasia	1/5	5/5	2/5	1/5
Atelectasis	4/5	4/5	4/5	4/5
Interstitial pneumonia	3/5	2/5	2/5	3/5

Table 6.3.1-16: 28–day Dietary Study in Wistar Rats. Test Compound Technical Glyphosate (FSG 03090 H/05 March 1990) ([redacted] , 1991): Summary incidence of histopathological findings

Findings	Dose level [ppm]			
	Males		Females	
	0	20000	0	20000
Perivascular leucocytic infiltration	0/5	1/5	1/5	0/5
Perivascular lymphocytic aggregation	4/5	3/5	5/5	4/5
Kidneys				
Intestial nephrosis	1/0	0/0	0/0	0/0
Spleen				
Lymphoid hyperplasia	2/5	0/5	0/5	1/5

III. CONCLUSIONS OF THE STUDY REPORT

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. However, there were a few incidences of urine incontinence in the mid- and high-dose groups but not in the high-dose recovery group. There were no notable intergroup differences in body weights, food consumption, or haematological parameters. With regards to clinical chemistry parameters, there was a statistically significant increase in blood urea nitrogen (BUN) level and in the activity of glutamic pyruvic transaminase (SGPT) at the high-dose level. These changes were not evident in the 14 day recovery group. There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

According to the 2nd amendment to the study report created in November of 1994, the NOAEL was established at 2000 ppm due to the lack of treatment-related effects.

Assessment and conclusion by applicant:
In this 4–week toxicity study, groups of five male and five female Wistar rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm (equivalent to 0, 17.6, 178.5, 1894.9 or 1987.5 mg/kg bw/day in males and 21.6, 223.3, 2250.8 or 2129.7 mg/kg/day in females). A high-dose recovery group was administered 20000 ppm for 28 days and then were followed for 14 days without treatment before being sacrificed. The study was conducted according to OECD 407 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

The study was well conducted, in compliance with GLP and OECD 407 (1981) and thus, can provide useful information for the assessment of repeated dose toxicity of glyphosate. The study is therefore considered valid.

Dosing Wistar rats via the diet with glyphosate produced slight increases in blood urea nitrogen (BUN) and glutamic pyruvic transaminase (SGPT) at the high-dose; these changes did not show a clear dose-response relationship and were shown to be reversible during the recovery period. There were no treatment-related effects at any dose level with regards to mortality, clinical signs of toxicity, body weight, haematology, organ weight and gross and histopathological findings. Based on this information, the no observed adverse effect level is determined to be the mid dose level 2000 ppm (178.5 mg/kg bw/day in males and 223.3 mg/kg bw/day in females).

Assessment and conclusion by RMS:

In disagreement with the previous assessment and the NOAEL proposed by the applicant, the RMS considers the effects reported at the top dose of 2000 ppm as not adverse. The increased glutamic pyruvic transaminase (SGPT/ALAT) levels in males and females at 20000 ppm were increased by 40-45% compared with controls, which is below the cut-off of 50% as proposed by JMPR as a starting point of adversity. In addition, the increase in blood urea nitrogen (BUN) was not considered treatment-related due to a lack of a clear dose-response relationship.

It should be noted that the study is considered acceptable but with restrictions (reliable with restrictions) as there are indications of lung infections based on the high on the incidence of lung congestion, interstitial pneumonia etc among both control and treated animals.

B.6.3.1.2. Oral 28-day study in rat - study 2

Data point	CA 5.3.1/004
Report author	[redacted]
Report year	1991
Report title	Glyphosate: 4 Week Dietary Toxicity Study in Rats
Report No	5626
Document No	Not reported
Guidelines followed in study	OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	Dose levels exceed the 1000 mg/kg bw/day limit dose. Reticulocytes, platelet count, total cholesterol, urea and bile acids not assessed. T3, T4 and TSH were not determined. Urinalysis not performed. Only liver, kidneys, adrenals, testes, epididymides were weighed. Only liver, heart, kidneys (in males), spleen and adrenals from the control and high dose group and kidney from all dose groups in females were examined histopathologically.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable with the restriction that the organ weight measurements and the histopathological investigation did not include all required organs and that urinalysis has not been performed.

Executive Summary

In a 4–week toxicity study, groups of five male and five female Sprague-Dawley rats were administered technical glyphosate for 28 consecutive days via the diet at concentrations calculated to achieve dose levels of 0 (control), 50, 250, 1000 or 2500 mg/kg bw/day.

General clinical observations were done daily and detailed clinical examinations weekly. Body weights and food consumption were assessed in weekly intervals. Water consumption was monitored by visual inspection throughout the study. Haematological, blood biochemistry parameters were evaluated during week 4 of dosing. At the end of the scheduled period, the animals were killed and subjected to a full post-mortem examination and selected organs were weighed and tissues were taken for subsequent histo pathology examination.

There was one premature death, an intermediate dose group male that died during the week 4 blood sampling. Incidences of soft faeces were noted in high dose males during weeks 3 and 4. There were no notable intergroup differences in body weight, food consumption, water consumption, or haematological parameters. As body weight gain was decreased with 11% compared with controls in top dose females, this is considered treatment-related and

adverse by the RMS. There were equivocal increases in alanine aminotransferase (ALT) (males at 250, 1000 and 2500 mg/kg bw/day; females at 2500 mg/kg bw/day), alkaline phosphatase (males at 250, 1000 and 2500 mg/kg bw/day; females at 1000 and 2500 mg/kg bw/day) and total bilirubin (females at 2500 mg/ kg bw/day), all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate. Plasma phosphate was slightly increased in 1000 and 2500 mg/kg bw/day males. At the top dose, the RMS considers the increased alkaline phosphatase levels in males and females and bilirubin levels in females as treatment-related and possibly adverse as changes are >50% compared with controls. The significant increase in phosphate levels in males at 1000 mg/kg bw/day and above is not considered adverse as it is not accompanied by other correlating effects.
There were no notable intergroup differences in organ weights. No gross pathology findings attributed to administration of glyphosate were recorded. Histopathological changes were limited to nephrocalcinosis in 250, 1000 and 2500 mg/kg bw/day females. The increases in plasma phosphate and nephrocalcinosis were considered expected findings in view of the dose levels employed and the phosphate content of glyphosate and are considered to be of unclear toxicological relevance.

The RMS disagrees with the NOAEL of 2500 mg/kg bw/day as proposed by the GRG. The RMS proposes a NOAEL of 1000 mg/kg bw/day based on a decreased body weight gain in females, increased alkaline phosphatase in males, increased bilirubin in females, increased incidence of soft stool in males at 2500 mg/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch#:	161-JRJ-131-2
Purity:	99.5%
Stability of test compound:	Not indicated

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 4 weeks on arrival
Sex:	Male and female
Weight at dosing:	♂ group means 154 – 161 g; ♀ group means 108 – 115 g
Acclimation period:	8 days
Diet/Food:	SDS Rat and Mouse No.1 Expanded Fine Ground Diet, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	2 or 3 rats per suspended polypropylene cage with stainless steel wire grid top and bottom.
Environmental conditions:	Temperature: 18 – 22 °C Humidity: 55 ± 10% Air changes: 15 – 20/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1988-12-21 to 1989-01-18

Animal assignment and treatment:
Groups of five male and five female Sprague-Dawley rats were administered technical glyphosate diet for 28 consecutive days via the diet at concentrations calculated to achieve dose levels of 0 (control), 50, 250, 1000 or 2500 mg/kg bw/day.

Table 6.3.1-1: Glyphosate: 4 Week Dietary Toxicity Study in Rats [redacted], 1991): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	5	5
Low	50	5	5
Intermediate I	250	5	5
Intermediate II	1000	5	5
High	2500	5	5

The test diets were prepared weekly by direct admixture of test material to untreated diet and blending for 20 minutes in a diet mixer. Samples of diets prepared for week 1 of the study were analysed with regard to stability, concentration and homogeneity. Data proving 21-day stability of glyphosate were generated prior to commencement of the study. The concentration of glyphosate in the diet was adjusted weekly in order to achieve a constant dose level in mg of test material per kg of the animal's body weight per day.

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
General clinical observations were done daily and detailed clinical examinations weekly.

Body weight
Body weights were assessed during the week prior to starting treatment and in weekly intervals thereafter.

Food consumption and utilisation
Food consumption was assessed in weekly intervals. Water consumption was monitored by visual inspection throughout the study.

Ophthalmoscopic examination
Ophthalmoscopic eye examinations were not performed.

Haematology and clinical chemistry
Blood samples from all rats were taken during week 4 dosing from the retro-orbital sinus under light anaesthesia. These samples were submitted for haematological and clinical chemistry examination. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell (RCB) count, leucocyte count and differential and coagulation via tail snip.

For clinical chemistry analysis the following parameters measured: Glucose, blood urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, creatinine, calcium, phosphate, sodium, potassium, chloride, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin and plasma and RBC cholinesterase.

Sacrifice and pathology
After 28 of consecutive treatment, all surviving animals were sacrificed via carbon dioxide followed by exsanguination and subjected to a gross pathological examination. The following organ weights were determined: Adrenals, kidneys, liver, and testes and epididymides.

Tissue samples were taken from the following organs and preserved: all gross lesions, adrenals, heart, kidneys, liver, ovaries (with fallopian tubes), spleen, testes (plus epididymides). Liver, heart, kidneys, spleen and adrenals

from the control and high dose group were examined histopathologically. Kidneys from the intermediate I, Intermediate II and low dose female groups were also subjected to histopathological evaluation.

Statistics
Haematology, clinical chemistry, organ weight and body 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 comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous, then transformations such as log and rank were used to achieve homogeneity, or a non-parametric test such as Kruskal-Wallis ANOVA was used. Organ weights were also analysed conditional on body weight (i.e. analysis of covariance). Histopathology data were analysed by Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was one premature death, a male from the 250 mg/kg bw/day dose group that died during the blood sampling in week 4. This was regarded as unrelated to test substance administration.

B. CLINICAL OBSERVATIONS
Three out of 5 high dose males had soft faeces during weeks 3-4 or in week 4 only. There were no other effects observed in males or females from the other dose groups considered related to treatment.

C. BODY WEIGHT
The 2500 mg/kg bw/day dose group showed a slight but consistent reduction in body weight gain throughout the dosing period. This reduction did not reach statistical significance, but was above 10% and therefore considered to be treatment related and adverse by the RMS. The 2500 mg/kg bw/day dose female group showed a slight but consistent reduction in body weight gain throughout the four weeks of dosing. The difference did not attain statistical significance. Results are presented in the following table:

Table 6.3.1-2: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted] 1991): Intergroup comparison of mean body weights and body weight gain

Dose level [mg/kg bw/day]	Initial body weight [g] Week 0	Final body weight [g] Week 4	Weight gain [g]	Weight gain [% of controls]
Males				
0	154	351	197	-
50	↑158	↑368	↑210	↑107
250	↑157	↑376	↑219	↑111
1000	↑161	↓349	↓188	↓95
2500	↑157	↓336	↓179	↓91
Females				
0	115	228	113	-
50	↓109	↓214	↓105	↓93
250	↓110	↑234	↑124	↑110
1000	↓108	↓212	↓104	↓92
2500	↓113	↓214	↓101	↓89 (-11%)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no notable intergroup differences in either sex. All groups receiving glyphosate performed similarly to if not better than their respective controls.

The concentration of glyphosate in the diet was adjusted weekly in order to achieve a constant dose level in mg of test material per kg of animal's body weight per day. Test compound intakes are presented in the following table:

Table 6.3.1-3: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted], 1991): Test compound intake

Test group	Dietary concentration [mg/kg bw/day]	Achieved dietary concentration [mg/kg bw/day]	
		Males	Females
Control	0	0	0
Low	50	49 ± 5.8	55 ± 7.9
Intermediate I	250	255 ± 9.6	277 ± 17.1
Intermediate II	1000	1034 ± 58.2	1047 ± 86.4
High	2500	2592 ± 226.9	2614 ± 318.4

E. OPHTHALMOSCOPIC EXAMINATION

Specific eye examinations were not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group.

Blood clinical chemistry

In males, there was a mild equivocal increase in alanine aminotransferase (ALT) of +21 %, +34 %, +25 % and an increase in alkaline phosphatase (AP) of +35 %, +23 %, +60 % in the 250, 10000 and 2500 mg/kg bw/day groups, respectively. Phosphate was also slightly increased in the 1000 mg/kg bw/day (+13 %) and 2500 mg/kg bw/day (+16 %) dose groups.

In females, ALT was significantly increased in the 2500 mg/kg bw/day group (+31 %), as was total bilirubin (+63 %). Alkaline phosphatase was increased in the 1000 and 2500 mg/kg bw/day dose groups but did not reach statistical significance and there was no relationship to dose level of Glyphosate. There were slight increases in sodium in the 50, 1000 and 2500 mg/kg bw/day dose groups (all +1 %) and in chloride in the 50 mg/kg bw/day group (+2 %) but these were considered due to chance (see table below):

Table 6.3.1-4: Glyphosate: 4 Week Dietary Toxicity Study in Rats ([redacted], 1991): Intergroup comparison of selected clinical chemistry parameters (mean ± SD)

Parameter	Dose level [mg/kg bw/day]				
	0	50	250	1000	2500
Males					
ALT [U/L]	68 ± 7	↑71 ± 13	**↑82* ± 8**	**↑91** ± 11**	**↑85* ± 9**
AP [U/L]	575 ± 84	↑624 ± 66	**↑779* ± 217**	↑707 ± 59	**↑921*** ± 162 (+60 %)**
Total bilirubin [µmol/L]	0.9 ± 0.3	↑1.0 ± 0.4	0.9 ± 0.5	↓0.8 ± 0.2	↑1.0 ± 0.4
Phosphate [mmol/L]	2.44 ± 0.20	↓2.24 ± 0.16	↑2.64 ± 0.17	**↑2.76** ± 0.19**	**↑2.82** ± 0.15**
Sodium [mmol/L]	145 ± 1	145 ± 1	↑146 ± 1	↑146 ± 2	145 ± 1
Chloride [mmol/L]	97 ± 1	↑98 ± 1	↑98 ± 2	↑98 ± 1	↑99 ± 1
Females					
ALT [U/L]	59 ± 13	↓52 ± 8	↑74 ± 13	↑67 ± 14	**↑77* ± 12**
AP [U/L]	485 ± 87	↑492 ± 184	↓469 ± 103	↑701 ± 79	↑687 ± 289 (+42%)
Total bilirubin [µmol/L]	0.8 ± 0.2	0.8 ± 0.2	↑1.1 ± 0.2	↑1.0 ± 0.2	**↑1.3** ± 0.3 (+63%)**
Phosphate [mmol/L]	2.28 ± 0.30	↓2.19 ± 0.10	↑2.38 ± 0.18	↑2.46 ± 0.22	↑2.32 ± 0.20
Sodium [mmol/L]	144 ± 1	**↑145** ± 1**	↑145 ± 1	**↑145** ± 1**	**↑145* ± 1**
Chloride [mmol/L]	98 ± 1	**↑100* ± 1**	98 ± 1	↑99 ± 2	**↑100** ± 1**

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$);
*** Statistically significant from controls ($p < 0.001$)

G. NECROPSY

Organ weights

There were no intergroup differences in either sex.

Gross pathology

There were no findings attributed to administration of glyphosate.

Histopathology

Findings related to glyphosate exposure were limited to the kidneys of females. Very mild to mild nephrocalcinosis (mineral deposition) was noted at the corticomedullary junctions in 2/5 of the 250 mg/kg bw/day females (RMS: one graded very mild, one as mild), 2/5 of the 1000 mg/kg bw/days females (RMS: one graded very mild, one as mild) and 4/5 of the 2500 mg/kg/day females (RMS: two graded as very mild, two graded as mild). This finding was not present in the 50 mg/kg bw/day or control females (See Table below):

Table 6.3.1-5: Glyphosate: 4 Week Dietary Toxicity Study in Rats [redacted], 1991): Summary incidence of histopathological findings

Finding	Dose level [mg/kg bw/day]									
	Males					Females				
	0	50	250	1000	2500	0	50	250	1000	2500
Nephro-calcinosis	0/5	-	-	-	0/5	0/5	0/5	2/5	2/5	4/5

III. CONCLUSIONS

There was one premature death, an intermediate dose group male that died during the week 4 blood sampling. Incidences of soft faeces were noted in high dose males during weeks 3 and 4. There were no notable intergroup differences in body weight, food consumption, water consumption, or haematological parameters. There were equivocal increases in alanine aminotransferase (ALT) (males at 250, 1000 and 2500 mg/kg bw/day; females at 2500 mg/kg bw/day), increases in alkaline phosphatase (males at 250, 1000 and 2500 mg/kg bw/day; females at 1000 and 2500 mg/kg bw/day) and total bilirubin (females at 2500 mg/kg bw/day), all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate. Plasma phosphate was slightly increased in 1000 and 2500 mg/kg bw/day males. There were no notable intergroup differences in organ weights. No gross pathology findings attributed to administration of glyphosate were recorded. Histopathological changes were limited to nephrocalcinosis in 250, 1000 and 2500 mg/kg bw/day females. The increases in plasma phosphate and nephrocalcinosis were considered expected findings in view of the dose levels employed and the phosphate content of glyphosate.

The No Effect Level (NOEL) was set at 50 mg/kg bw/day in the study report.

Assessment and conclusion by applicant:

In this study groups of male and female Sprague-Dawley rats were administered glyphosate via the diet at dose levels of 0, 50, 250, 1000 or 2500 mg/kg bw/day for 28 days according to OECD 407 (1981).

Dosing Sprague-Dawley rats via the diet with glyphosate produced slight increases in plasma phosphate levels in males (1000 and 2500 mg/kg bw/day) and increases in incidence of nephrocalcinosis in females (≥250, 1000 and 2500 mg/kg bw/day), both being expected findings in view of the dose levels employed and the phosphate content of glyphosate. In addition, there were equivocal increases in alanine aminotransferase (males at ≥250 mg/kg bw/day; females at 2500 mg/kg bw/day), alkaline phosphatase (males at ≥250 mg/kg bw/day; females at 1000 and 2500 mg/ kg bw/day) and total bilirubin (females at 2500 mg/kg bw/day), all of which were regarded as normal responses to increased liver activity at the elevated dose levels of glyphosate.

Since the effects observed were considered to be adaptive, the NOAEL was set at 2500 mg/kg bw/day for male and female Sprague-Dawley rats under the conditions of this study.

Assessment and conclusion by RMS: The RMS disagrees with the NOAEL of 2500 mg/kg bw/day as proposed by the GRG. The RMS proposes a NOAEL of 1000 mg/kg bw/day based on a decreased body weight gain in females, increased alkaline phosphatase in males, increased bilirubin in females and soft stool in males at 2500 mg/kg bw/day.
Histopathological changes were limited to nephrocalcinosis in 250, 1000 and 2500 mg/kg bw/day females (grade very mild to mild) which was considered to be of unclear toxicological relevance. Further, the significant increase in phosphate levels in males at 1000 mg/kg bw/day and above is not considered adverse as it is not accompanied by other correlating effects.

In the previous assessment in the DAR, a NOEL of 50 mg/kg bw/day was derived based on increased ALAT, increased alkaline phosphatase, increased bilirubin, increased phosphate on increased incidence of nephrocalcinosis in females. No additional comments on this study were made in the RAR (2015).

B.6.3.1.3. Oral 28-day in rat - study 3

Data point	CA 5.3.1/005
Report author	[redacted]
Report year	1989
Report title	Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats
Report No	[redacted] 8921
Document No	Not reported
Guidelines followed in study	Not reported; in general compliance to OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	Temperature-range of the housing conditions was below 22 ± 3 °C; no sensory reactivity tests were performed; no haematology/clinical chemistry were performed; no organ weights were determined; histopathology was performed on liver and kidneys only. Dose levels exceed the 1000 mg/kg bw/day limit dose. Thyroid hormone levels were not determined.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Supportive, category 3b **Conclusion AGG**: The study is considered to be acceptable but with restrictions (reliable with restrictions) as this is designed as a dose-range finding study with limited parameters included (refer to deviations above)

Executive Summary
Glyphosate (Lot XLI-203; 97.67% pure) was administered in the diet to four groups of five male and five female Charles River Sprague-Dawley rats at target concentrations of 0, 30000, 40000 or 50000 ppm (equivalent to 0, 1921.1, 2634.1 or 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 or 4150.2 mg/kg bw/day for females) for four weeks. Food and water were available *ad libitum*. Diets were prepared once at the beginning of the study.
All animals were observed twice daily for mortality and moribundity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. All animals were sacrificed and given a gross necropsy at the end of the study. The liver and kidneys from each animal were preserved; these organs were examined microscopically for control and high dose animals.

No animals died during the course of the study. Reduced body weight gains were noted in both sexes at all three dose levels, which was considered treatment-related and adverse by the RMS. Food consumption (g/day) was reduced for mid and high dose males during the first week of the study. Food intake for treated females was comparable to controls throughout the study. The only clinical signs of toxicity were soft stool and/or diarrhoea,

which occurred in both sexes at all dose levels; nine-of-ten low dose animals had loose stools, all mid dose rats had loose stools and/or diarrhoea, while diarrhoea was the predominant sign in high dose animals during the last three weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (T880068)
Description:	White powder
Lot/Batch#:	XLI-203
Purity:	97.67%
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Albino rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Ca. 9 weeks
Sex:	Male and female
Weight at dosing:	♂ 259.9 – 340.3 g; ♀ 187.7 – 237.2 g
Acclimation period:	27 days
Diet/Food:	Purina Mills Certified RODENT CHOW # 5002, *ad libitum*
Water:	Water (St. Louis public water supply), *ad libitum*
Housing:	Individual suspended stainless steel cages
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 40 – 70% Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1988-07-25 to 1988-08-22

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 5 Sprague-Dawley rats per sex for a minimum of 28 days. Dietary concentrations were 0, 30000, 40000 and 50000 ppm (equivalent to 0, 1921.1, 2634.1 and 3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 and 4150.2 mg/kg bw/day for females).

Table 6.3.1-1: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Study design

Test group	Dietary concentration [ppm]	Average dietary concentration [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	5	5
Low	30000	♂: 1921.1; ♀: 2310.6	5	5
Mid	40000	♂: 2634.1; ♀: 3256.4	5	5
High	50000	♂: 3278.1; ♀: 4150.2	5	5

Preparation of the test diet
Purina Mills Certified RODENT CHOW #5002 was mixed for 10 minutes using a Hobart HCM-450 mixer. A batch size of 12 kg per dose was produced once during this study.

Mortality
Animals were checked twice daily for mortality and moribundity.

Clinical observations
Detailed observations for clinical signs of toxicity were performed weekly.

Body weight
The weight of each animal was recorded once weekly.

Food consumption
The quantity of food consumed by each animal was recorded once each week.

Sacrifice and pathology
All animals were killed and necropsied. External and internal investigations were performed on opened internal cavities and organs *in situ* and then removed. Hollow organs were opened and examined.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals. The following tissues were investigated: Kidneys and liver.

Statistics
The following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls:
Dunnett's Multiple Comparison Test (two-tailed): Body weights, food consumption
Fisher's Exact Test with Bonferroni Inequality Procedure: Incidence of microscopic lesions
Other statistical routines used for some data were: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubbs' Test to detect outliers.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES
Please refer to the table above Table 6.3.1.3-1.

B. MORTALITY
There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS
Clinical signs that were considered related to treatment included soft stool and/or diarrhoea, and were seen in animals of both sexes at all three dose levels.
Frequently, the lowest dose level animals had soft stools (5/5 males and 4/5 females) and the middle dose level animals had soft stools (5/5 males and 5/5 females) and diarrhoea (4/5 males and 5/5 females). The highest dose level animals experienced incidences of soft stool (4/5 males and 5/5 females) and marked diarrhoea (5/5 males and 5/5 females). No other treatment-related clinical signs were noted.

D. BODY WEIGHT
Slightly reduced body weight gains occurred for both sexes at all three dose levels. However, differences in body weights between treated and control groups were less than 10 %. This should be set in context to the fact that the dietary inclusion levels represented 3 %, 4 % and 5 % by mass of the feed offered, and consequently the calorific value of the feed was concomitantly reduced.

Table 6.3.1-2: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Body weight means [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Study mean	360.9	↓355.0	↓342.9	↓335.0	234.2	↓225.2	↓225.9	↓219.8
% Difference from control (study mean)	-	-1.7	-5.0	-7.2	-	-3.8	-3.6	-6.2
% Difference from control (final body weight)	-	-2.3	-5.9	-9.6	-	-5.9	-4.5	-9.0
% Gain from initial	27.2	↓22.9	↓20.8	↓15.4	23.1	↓15.4	↓17.8	↓12.6

Table 6.3.1-3: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Summary of body weight data [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Pretest	313.3 ± 24.25	↑316.6 ± 20.59	↓310.6 ± 30.37	↓312.0 ± 19.24	206.6 ± 15.32	↑207.2 ± 18.09	↓206.1 ± 14.72	205.6 ± 16.02
Day 6	337.7 ± 28.26	↓332.6 ± 21.14	↓322.0 ± 39.20	↓311.0 ± 26.37	224.8 ± 14.34	↓217.6 ± 17.22	↓216.1 ± 13.47	↓212.2 ± 14.98
Day 16	372.0 ± 30.40	↓360.4 ± 19.92	↓345.8 ± 46.67	↓341.2 ± 27.13	240.0 ± 15.27	↓231.0 ± 18.44	↓230.0 ± 16.64	↓223.4 ± 18.70
Day 23	383.0 ± 32.10	↓375.5 ± 21.05	↓361.0 ± 47.99	↓350.7 ± 31.36	245.4 ± 16.88	↓231.1 ± 17.27	↓234.5 ± 12.54	↓226.3 ± 18.16
Day 28	398.6 ± 35.77	↓389.5 ± 21.79	↓375.1 ± 48.90	↓360.2 ± 35.90	254.3 ± 16.93	↓239.2 ± 19.29	↓242.8 ± 19.85	↓231.5 ± 20.93

Table 6.3.1-4: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Summary of cumulative body weight changes [g]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Day 1 – 8	24.3 ± 6.21	↓15.8 ± 5.62	**↓11.4* ± 11.23**	**↓-1.0** ± 7.27**	18.2 ± 5.70	↓10.3 ± 7.83	↓10.0 ± 7.18	**↓6.6* ± 4.52**
Day 1 – 16	58.6 ± 8.38	↓43.6 ± 11.90	**↓36.2* ± 16.35**	**↓29.2** ± 9.00**	33.4 ± 10.54	↓23.8 ± 6.12	↓23.9 ± 5.87	**↓17.8** ± 4.76**
Day 1 – 23	69.6 ± 6.89	↓58.7 ± 8.48	↓50.4 ± 17.91	**↓38.7** ± 12.49**	38.8 ± 10.73	**↓23.9* ± 7.14**	↓28.3 ± 8.18	**↓20.7** ± 6.46**
Day 1 – 28	85.3 ± 12.31	↓72.7 ± 6.98 (-15%)	↓64.5 ± 19.13 (-24%)	**↓48.2** ± 16.94 (-43%)**	47.7 ± 7.51	**↓32.0* ± 9.10 (-23%)**	↓36.7 ± 12.08 (-23%)	**↓25.8** ± 7.07 (-46%)**

* Statistically significant from control (Dunnett's test; $p \leq 0.05$);
** Statistically significant from control (Dunnett's test; $p \leq 0.01$)

E. FOOD CONSUMPTION

Food consumption was reduced on a g/day basis for males at the two highest dietary levels as compared to their respective controls during the first week of testing.

After that, food consumption was comparable to that of the control animals during the remainder of the study. Food intake for females was comparable to control throughout the study.

Table 6.3.1-5: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Food consumption [g/day]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Day 1 – 8	23.6 ± 1.98	↓20.9 ± 2.18 (11%)	**↓19.5* ± 2.88 (17%)**	**↓17.0** ± 1.92 (28%)**	18.5 ± 1.63	↓15.7 ± 0.69 (15%)	↓16.0 ± 1.93 (14%)	↓16.8 ± 4.46 (9%)
Day 8 – 16	24.3 ± 1.98	↓24.0 ± 1.50	↓23.9 ± 3.29	↓23.0 ± 0.96	18.8 ± 2.34	↓17.4 ± 0.86	↓18.5 ± 2.32	↓17.9 ± 0.90
Day 16 – 23	24.6 ± 2.21	↑25.0 ± 0.99	↑24.7 ± 3.38	↑25.5 ± 0.65	19.1 ± 1.24	↓18.1 ± 0.43	↑19.5 ± 2.12	↓18.8 ± 1.65
Day 23 – 28	23.8 ± 2.42	↓23.5 ± 0.66 (1%)	↑24.5 ± 3.06 (3%)	↑24.3 ± 2.31 (2%)	20.7 ± 3.92	↓17.1 ± 0.74 (17%)	↓18.2 ± 2.73 (12%)	↓18.6 ± 0.37 (10%)

* Statistically significant from control (Dunnett's test; $p \leq 0.05$);
** Statistically significant from control (Dunnett's test; $p \leq 0.01$)

Table 6.3.1-6: Range-finding Study of Glyphosate Administered in Feed to Sprague-Dawley Rats ([redacted] 1989): Mean food consumption [g/day]

	Dose group [ppm]							
	Males				Females			
	0	30000	40000	50000	0	30000	40000	50000
Study mean	24.1	23.4	23.2	22.5	19.3	17.1	18.1	18.0
% Difference from control (study mean)	n.a.	-2.9	-3.7	-6.6	n.a.	-11.4	-6.2	-6.7

n.a.: not applicable

F. NECROPSY

Gross pathology

There were no treatment-related changes observed at necropsy.

Histopathology

There were no microscopic lesions related to treatment.

III. CONCLUSIONS

No animals died during the course of the study. Slightly reduced body weight gains were noted in both sexes at all three dose levels, although significant reductions consistently occurred only in high dose males and females (9.6 and 9.0 %, respectively, after 4 weeks). Food consumption (g/day) was reduced for mid and high dose males during the first week of the study. Food intake for treated females was comparable to controls throughout the study. The only clinical signs of toxicity were soft stool and/or diarrhoea, which occurred in both sexes at all dose levels; nine-of-ten low dose animals had loose stools, all mid dose rats had loose stools and/or diarrhoea, while diarrhoea was the predominant sign in high dose animals during the last three weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions.

According to the study author, the body weight reduction may have been secondary to the described gastrointestinal effects, rather than a direct toxic response to treatment. This interpretation was supported by the absence of any corresponding changes in food intake, survival, gross and/or microscopic pathology.

Assessment and conclusion by applicant:
In this study, glyphosate was administered in the diet to four groups of five male and five female Sprague-Dawley rats at target concentrations of 0, 30000, 40000 or 50000 ppm (equivalent to 0, 1921.1, 2634.1 or

3278.1 mg/kg bw/day for males and 0, 2310.6, 3256.4 or 4150.2 mg/kg bw/day for females) for four weeks in general compliance to OECD 407.

Significant incidences of soft stools and/or diarrhoea were noted for both sexes at all three exposure levels with the highest dose group most affected. Slightly reduced body weight gains occurred for both sexes at all three dose levels but were at all times less than 10 % of the corresponding controls, and were probably in part due to the lower calorific value of the diet offered (due to high level of glyphosate inclusion). This body weight reduction may have been secondary to the observed gastrointestinal effects. This interpretation is supported by the absence of any corresponding changes in food intake, survival, gross or microscopic pathology. No other significant effects were noted in this study.

Selection of dose levels for a longer term study should consider secondary effects (nutritional imbalance, dehydration and death) often related to diarrhoea.

Based on the results of this 28–day range-finding study, no robust NOAEL can be derived.

Assessment and conclusion by RMS: The RMS agrees with the assessment of the GRG, except with the conclusion that body weight gain was only slightly reduced (within 10% compared with the corresponding controls). Based on the mean body weight gains presented in Table 6.3.1.3-4, the RMS considers that body weight gain was adversely affected in all dose groups for at all observation intervals and for both males and females (>10% compared with controls). In addition, the RMS disagrees with the statement by the study authors and GRG that the body weight reduction is secondary to the described gastrointestinal effects, rather than a direct toxic response to treatment as other parameters that were included in the study were rather limited (organ weights, histopathology).

The conclusion that no robust NOAEL can be derived is in line with the previous assessments in the DAR and RAR (2015) on glyphosate.

B.6.3.1.4. Oral 28-day in mice

Data point	CA 5.3.1/006
Report author	[redacted]
Report year	1978
Report title	A Four Week Pilot Study with Glyphosate in Mice
Report No	77-2110
Document No	Not reported
Guidelines followed in study	No guideline followed, similar to OECD 407 (1981)
Deviations from current test guideline (OECD 407, 2008)	No sensory reactivity was investigated; no haematology or clinical chemistry was performed; organ weights were not determined; histopathology was not performed.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP).
Acceptability/Reliability	**Conclusion GRG**: Supportive, category 3b **Conclusion AGG**: The study is considered to be acceptable but with restrictions (reliable with restrictions) (as a dose range finding study) as this study is designed as a non-guideline dose-range finding study with very few parameters included (refer to deviations above)

Executive Summary

This study was conducted to select dose levels for a 90–day sub-chronic study of glyphosate. The test substance was administered orally via the diet to mice at target dose levels of 0 (control), 100, 300 or 1000 mg/kg bw/day (approximately 80, 235 or 800 mg/kg bw/day actual achieved dose, after correction for test substance purity). All survivors were necropsied after four weeks of test substance administration.

Animals were observed for mortality, clinical signs, body weight changes, food consumption and gross pathology.

All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects at any of the dose levels administered.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	Course white powder
Lot/Batch#:	XHI-162
Purity:	83%
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Mouse
Strain:	CD-1, COBS (ICR derived)
Source:	[redacted]
Age:	7 weeks
Sex:	Male and female
Weight at dosing:	♂ 28 g (25 – 31 g); ♀ 22 g (20 – 24 g)
Acclimation period:	18 days
Diet/Food:	Standard laboratory diet (Purina Laboratory Chow®) *ad libitum*; fresh food presented twice weekly.
Water:	Water, *ad libitum*
Housing:	Individually in elevated stainless steel wire mesh cages.
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported

B: Study design and methods

In life dates: 1978-03-13 to 1978-04-12

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 5 CD-1 mice per sex for 30 days. Dietary concentrations were 0, 100, 300 and 1000 mg/kg bw/day (approximately 80, 235 and 800 mg/kg bw/day after correction for test substance purity).

Table 6.3.1-1: A Four Week Pilot Study with Glyphosate in Mice [redacted] 1978): Study design

Test group	Target dietary concentration [mg/kg bw/day][1]	Achieved compound intake [mg/kg bw/day]	Males	Females
Control	0	0	5	5
Low	100	80	5	5
Mid	300	235	5	5
High	1000	800	5	5

[1] Active substance-dosing based on 98.5 % activity of the technical material. Upon analysis of the test material by the sponsor, glyphosate was assayed at 83 % active.

Analysis of the test diet
Appropriate amounts of compound (adjusted by most recent weekly body weight and food consumption data) and standard laboratory diet were mixed weekly. A 4 oz. sample of each dietary level plus control diet was taken from each weekly batch of diet prepared. In addition, when feeders were filled, two extra feeder jars of each dietary level plus control feed were placed in an empty animal cage immediately adjacent to the last animal in each group and sampled after 7 days. All feed samples (the 4 oz. samples taken at each diet preparation from each batch as well as the entire contents of the two extra feeders for Groups I – IV) were stored frozen at [redacted]. Samples were packed on dry ice and sent to [redacted] for analysis on 18 April 1978.

Mortality
Mice were checked twice daily for mortality.

Clinical observations
All animals were examined for gross signs of toxicological or pharmacological effects twice daily. Additionally, all animals received a detailed physical examination for signs of local or systemic toxicity and pharmacological effects once each week.

Body weight
The weight of each animal was recorded twice prior to treatment, weekly during treatment and terminally (after fasting).

Food consumption and compound intake
The quantity of food consumed by each animal was recorded once prior to treatment and weekly during treatment. Please refer to the table above for information on the compound intake.

Sacrifice and pathology
All animals were killed by exsanguination under ether anaesthesia. Tissues with gross abnormalities were preserved in 10 % neutral buffered formalin and held for possible future examination.

Statistics
Body weight and food consumption data were analysed. Mean values of all dose groups were compared to control at each time interval.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF THE FORMULATED DIETS
The analysis of the formulated diets was in the responsibility of the sponsor.

B. MORTALITY
All animals survived the duration of the study.

C. CLINICAL OBSERVATIONS
There were no physical observations noted which were considered related to the administration of the test material.

D. BODY WEIGHT

Slightly lower mean body weights, as compared to control, were noted for males receiving 300 or 1000 mg/kg bw/day through week two. This only achieved statistical significance during the first week in high dose animals. The high dose males has slightly lower mean body weight pre-test and as a consequence. These differences were not considered related to the administration of Glyphosate.

Mean body weights for males receiving 100 mg/kg bw/day and all test substance-treated females were considered comparable to those of the controls throughout the course of the study.

Table 6.3.1-2: A Four Week Pilot Study with Glyphosate in Mice (1978): Summary of body weight data [g]

Week	Dose group [mg/kg bw/day]							
	Males				Females			
	0	100	300	1000	0	100	300	1000
-1	24.8 ± 1.6	24.8 ± 1.8	↑25.4 ± 1.8	↓24.4 ± 0.9	19.4 ± 0.5	19.4 ± 0.5	↓19.2 ± 0.8	↑19.6 ± 0.9
0	29.8 ± 0.8	↓28.4 ± 2.2	↓27.8 ± 1.9	**↓27.6* ± 1.8**	21.6 ± 0.9	↓21.2 ± 0.8	↑22.4 ± 1.1	↑21.8 ± 1.4
1	32.6 ± 1.5	↓31.6 ± 1.7	↓31.0 ± 1.6	↓31.4 ± 1.7	23.8 ± 0.8	↑24.2 ± 1.1	↑24.4 ± 0.9	↑24.6 ± 1.5
2	33.8 ± 1.3	↓32.6 ± 2.3	↓32.0 ± 2.0	↓31.8 ± 2.0	25.2 ± 0.8	↑25.6 ± 1.5	↑26.0 ± 1.4	↑26.6 ± 2.1
3	33.2 ± 1.6	↑33.6 ± 2.2	↑33.4 ± 1.9	33.2 ± 1.9	26.4 ± 1.5	↓25.2 ± 1.6	↑26.6 ± 0.9	↑26.6 ± 1.8
4	34.4 ± 1.3	↓34.0 ± 2.3	↓33.6 ± 1.7	34.4 ± 1.9	28.2 ± 1.5	↓26.6 ± 1.8	↓27.2 ± 1.1	↓27.6 ± 2.2

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption values for all animals receiving Glyphosate were generally comparable to those of the controls throughout the study.

Appropriate amounts of test material, based on 98.5 % activity, were mixed fresh weekly in Purina Laboratory Chow® to yield dose levels of 100, 300 and 1000 mg of test material per kg of body weight per day. Upon analysis by the sponsor, glyphosate assayed at an activity of 83 %. Consequently, the mean compound consumption values are approximately 80 % of the proposed dose levels.

F. NECROPSY

Gross pathology

Macroscopic post mortem observations did not reveal any changes considered related to the administration of test material.

III. CONCLUSIONS

All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects at any of the dose levels administered.

Assessment and conclusion by applicant:

In this range-finding, the test substance glyphosate was administered orally via the diet to CD-1 mice at doses of approximately 80, 235 or 800 mg/kg bw/day of active substance similar to OECD 407 (1981).

All in-life data (physical observations, body weight and food consumption) and gross necropsy observations indicated no adverse effects of glyphosate at any of the dose levels administered.

Assessment and conclusion by RMS: The RMS agrees with the conclusion of the applicant. As the study is considered a dose-range finding study with limited reporting, no NOAEL has been derived.

This conclusion is in line with the previous assessments in the DAR and RAR (2015) on glyphosate.

B.6.3.1.5. 14-day dose range finding study in dogs

Data point:	CA 5.3.1/007
Report author	[redacted]
Report year	1989
Report title	Glyphosate: Oral Maximum Tolerated Dose Study in Dogs
Report No	5660
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA 82-1
Deviations from current test guideline:	This study was essentially a dose range-finding study for which no guideline is available.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The RMS considers this study as unacceptable as this study is designed as a non-guideline oral maximum tolerated dose study with only one dog per sex per treatment regime (Part A and Part B) and limited parameters were included in the study. No control animals were included in the study.

Executive Summary

This study was conducted to determine the oral maximum tolerated dose for glyphosate in Beagle dogs for dosing subsequent repeated dose toxicity studies in this species. Glyphosate was administered by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw daily (Part A) and to one dog of each sex for 14 days at 1000 mg/kg bw/day (Part B).

Clinical observations were made daily. Body weights were assessed twice weekly and food consumption recorded daily. For part A, laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and after 7 days of treatment with each dose level. For part B, blood samples were taken before dosing and after 14 days of dosing. Urine samples were collected pre-trial and the day prior to sacrifice (part B only). Samples were collected over the final 17 hours of a 21 hour period of water deprivation. Faecal analysis for occult blood was performed at the time of the urine collections. At the end of the scheduled dosing periods, the animals were sacrificed and subjected to a full examination *post mortem*. Selected organs were weighed and tissues were taken for possible subsequent histopathology examination that was not performed.

There were no mortalities. There were no clinical signs of toxicity considered treatment related after 21 days of dosing up to 1000 mg/kg bw/day (Part A). Other than loose faeces in the one female during the dosing period, there were no other clinical observations considered related to treatment during 14 days dosing at 1000 mg/kg bw/day (Part B). Body weight profiles or food consumption were not affected by administration of glyphosate. No haematological effects considered related to treatment were observed. A slight increase in alanine aminotransferase occurred in the two male dogs treated with glyphosate and a slight reduction in cholesterol levels in dogs of both sexes that were not considered treatment related. There were no effects of glyphosate administration on urine parameters or occult blood detected in faeces in dogs of either sex. At necropsy, there were no gross lesions observed attributable to treatment with glyphosate with dose escalation or 14 days dosing at 1000 mg/kg bw/day. There was a reduction in absolute and relative spleen weight in the male dog with dose escalation to 1000 mg/kg bw/day but not in the male dog treated with glyphosate for 14 days. Microscopic examination of tissues was not performed.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch#:	161-JRJ-131-2
Purity:	99.5 %
Stability of test compound:	Not indicated

2. Vehicle and/or positive control: No controls utilised.

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	4 months
Sex:	Male and female
Weight at dosing:	Part A: ♂ 8.5 kg ♀ 8.6 kg; Part B: ♂ 9.2 kg ♀ 9.3 kg
Acclimation period:	At least 2 weeks.
Diet/Food:	Special Diet Services (SDS) complete dry Diet A, 400 g/day approximately on hour after dosing.
Water:	Tap water, *ad libitum*
Housing:	Individually in pens
Environmental conditions:	Temperature: ~17 °C (14 – 22 °C transient extremes) Humidity: ~42 % (18 – 56 % transient extremes) Air changes: 15 per hour 12 hours light/dark cycle

B: Study design and methods

In life dates: Part A 1989-01-31 to 1989- 02-21; Part B 1989-02-28 to 1989-03-14

Animal assignment and treatment:
This study was essentially a dose range-finding study to determine the oral maximum tolerated dose of glyphosate for setting dose levels for subsequent chronic dog studies. Dose levels up to a maximum limit level of 1000 mg/kg bw/day were investigated. Dose levels were selected based on available acute toxicology data. The study was conducted in two parts, A and B. For part A, one male and one female dog were dosed orally by gelatin capsule at escalating dose levels of 100, 300 and 1000 mg/kg bw/day. Each dose level was administered for 7 consecutive days. For part B, one male and one female were dosed orally by gelatin capsule with 1000 mg/kg bw/day for 14 consecutive days. Daily multiple capsule dosing was necessary for the 1000 mg/kg bw/day dose level. One batch of the test material (Batch no. 161-JRJ-131-2, purity 99.5 %) was used during the course of the study. Kruger hard, clear gelatin capsules (35 × 17 mm) were used for the formulation of individual animal daily doses. Encapsulation of the bulk powder into individual animal daily doses was carried out weekly. Individual doses were calculated and prepared on the basis of the animals' most recently recorded body weight.

Table 6.3.1-1: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted] 1989): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Part A	100 (7 days)	1	1
	300 (7 days)		
	1000 (7 days)		
Part B	1000 (14 days)	1	1

Mortality
Each animal was checked for mortality or signs of morbidity during daily clinical observations.

Clinical observations
A check for clinical signs of toxicity at regular intervals throughout each working day, generally early morning and as late as possible each day.

Body weight
The body weight of each animal was recorded twice weekly.

Food consumption and utilisation
Individual food consumption was recorded daily.

Ophthalmoscopic examination
Ophthalmoscopy was not performed.

Haematology and clinical chemistry
For part A, laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and after 7 days of treatment with each dose level. For part B, blood samples were taken before dosing and after 14 days of dosing. All blood samples were taken from the jugular vein after the dogs had been fasted overnight.

EDTA was used as an anti-coagulant for evaluation of all haematology parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time.

For clinical chemistry evaluations, heparin was used as an anti-coagulant and plasma analysed for the following parameters: Blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, sodium, potassium, calcium, chloride, creatinine, total protein, protein electrophoresis, albumin, albumin/globulin ratio, cholesterol, alkaline phosphatase, phosphate and total bilirubin.

Urinalysis/Faecal analysis
Urine samples were collected (part B only) pretrial and the day prior to sacrifice using metabolism cages. Samples were collected over the final 17 hours of a 21 hour period of water deprivation. The following parameters were measured: Appearance, volume, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed for the presence of epithelial cells, white blood cells, red blood cells, crystals, casts, organisms and abnormal constituents. Faecal analysis for occult blood was performed at the time of the urine collections.

Sacrifice and pathology
After completion of the respective dosing periods, the animals were sacrificed by intravenous pentobarbitone sodium followed by exsanguination and subjected to a gross pathological examination. Any macroscopic findings were recorded. Terminal body weights were recorded.

The following organ weights were determined: heart, liver, kidneys and spleen.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, heart, kidneys, liver, ovaries, spleen, testes and thymus. These tissues were retained as a contingency for subsequent microscopic examination.

Statistics
Not applicable, only one animal per each sex used for each phase of the study and each animal was its own control.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Part A: There were no treatment related signs observed after 7 days dosing at 100, 300 or 1000 mg/kg bw/day.

Part B: Other than loose faeces in the female during the course of the dosing period, no treatment related signs were observed during the 14 days of dosing at 1000 mg/kg bw/day. The incidence of the loose faeces was not reported.

C. BODY WEIGHT
Part A: Body weight profiles were considered to be satisfactory for both dogs during the treatment period.

Part B: Body weight profiles were considered to be satisfactory for both dogs during the treatment period.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
Part A: There were no effects on food consumption from treatment with glyphosate.

Part B: There were no effects on food consumption from treatment with glyphosate.

E. OPHTHALMOSCOPIC EXAMINATION
Not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
Part A: There was a mild reduction in haemoglobin in the male with a mild increase in reticulocytes on Day 22 after the final dosing up to 1000 mg/kg bw/day that was considered related to the repeated blood sampling up to this study day. There were no findings considered to be treatment-related.

Part B: There were no treatment related effects in either dog after 14 days of dosing at 1000 mg/kg bw/day.

Table 6.3.1-2: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted] 1989): Selected haematology parameters (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
Haemoglobin [g/dL]						
Part A						
Pre-test	12.1			13.9		
Day 7	12.0			14.0		
Day 14		10.9			13.6	
Day 22			11.6			13.8
Part B						
Pre-test	12.9			14.2		
Day 22			13.9			15.1
Reticulocytes [%]						
Part A						
Pre-test	0.2			0.3		
Day 7	0.3			0.2		
Day 14		0.9			0.6	

Table 6.3.1-2: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted] 1989): Selected haematology parameters (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
Day 22			1.7			1.3
Part B						
Pre-test	1.2			1.5		
Day 22			1.2			0.6

Blood clinical chemistry
Part A: There were no effects considered related to treatment at the 3 dose levels. A mild increase in alanine aminotransferase (ALT) was observed in the male dog and cholesterol levels were slightly reduced in both male and female animals but these were not considered treatment related effects.

Part B: There were no treatment related effects. A mild increase in alanine aminotransferase was observed in the male dog but was not considered treatment related.

Table 6.3.1-3: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs ([redacted] 1989): Selected Clinical chemistry parameters (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
ALT [IU/L]						
Part A						
Pre-test	27			22		
Day 7	45			23		
Day 14		49			22	
Day 22			54			21
Part B						
Pre-test	44			23		
Day 22			60			30
Cholesterol [mmol/L]						
Part A						
Pre-test	4.3			4.8		
Day 7	3.7			4.2		
Day 14		3.6			4.1	
Day 22			3.7			4.0
Part B						
Pretest	3.6			4.0		
Day 22			3.6			3.1

G. URINE ANALYSIS/FAECAL ANALYSIS

Part A: Not performed.

Part B: There were no treatment related effects for urine and faeces were negative for occult blood.

H. NECROPSY

Organ weights
Part A: The absolute and relative weights of the spleen were reduced for the male dog. The absolute and relative weight of the spleen for the female and weights of other organs for both sexes were considered to be within normal historical ranges for dogs in this facility.

Part B: Values for the organs weighted were considered to be within normal limits.

Table 6.3.1-4: Glyphosate: Oral Maximum Tolerated Dose Study in Dogs [redacted] 1989): Spleen Weight - Absolute and relative to body weight (individual values)

Parameter	Dose Level [mg/kg bw/day]					
	Male			Female		
	100	300	1000	100	300	1000
Absolute spleen weight [g]						
Part A						
Day 22			26.91			47.75
Part B						
Day 22			56.21			41.94
Relative spleen weight [% of body weight]						
Part A						
Day 22			0.29			0.49
Part B						
Day 22			0.58			0.47

Gross pathology
Part A: No lesions attributable to treatment with glyphosate were detected.

Part B: No lesions attributable to treatment with glyphosate were detected.

Histopathology
Not performed for Parts A or B.

III. CONCLUSIONS

There were no mortalities. There were no clinical signs of toxicity considered treatment related after 21 days of dosing up to 1000 mg/kg bw/day (Part A). Other than loose faeces in the one female during the dosing period, there were no other clinical observations considered related to treatment during 14 days dosing at 1000 mg/kg bw/day (Part B). Body weight profiles or food consumption were not affected by administration of glyphosate. No haematological effects considered related to treatment were observed. A slight increase in alanine aminotransferase occurred in the two male dogs treated with glyphosate and a slight reduction in cholesterol levels in dogs of both sexes that were not considered treatment related. There were no effects of glyphosate administration on urine parameters or occult blood detected in faeces in dogs of either sex. At necropsy, there were no gross lesions observed attributable to treatment with glyphosate with dose escalation or 14 days dosing at 1000 mg/kg bw/day. There was a reduction in absolute and relative spleen weight in the male dog with dose escalation to 1000 mg/kg bw/day but not in the male dog treated with glyphosate for 14 days. Microscopic examination of tissues was not performed.

Based on these results it was concluded that the oral maximum tolerated dose of glyphosate in Beagle dogs is in excess of 1000 mg/kg bw/day. The report recommends, however, that the high dose level in any subsequent sub-chronic toxicity study should not exceed 1000 mg/kg bw/day as multiple capsule dose administration would be the limiting factor.

Assessment and conclusion by applicant:
In this study, glyphosate was administered by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw daily (Part A) and to one dog of each sex for 14 days at 1000 mg/kg bw/day (Part B). This study was essentially a dose range-finding study conducted in compliance with GLP regulations (no certificate of the competent authority was provided).

Oral administration of glyphosate by gelatin capsule to one male and one female dog for 7 day periods at escalating dose levels of 100, 300 and 1000 mg/kg bw/day produced no adverse effects. Fourteen days of treatment at 1000 mg/kg bw/day in an additional male and female dog was also well tolerated. There were no clinicopathological, gross pathological or organ weight findings considered attributable to treatment. It is unclear, however, if a slight increase in blood alanine aminotransferase in the two male dogs could potentially be related to treatment with glyphosate but this increase did not exceed twice the pretreatment levels in these dogs and thus would not be considered adverse.

Based on these results it is concluded that the oral maximum tolerated dose of glyphosate in Beagle dogs is greater than 1000 mg/kg bw/day.

Assessment and conclusion by RMS: The RMS agrees that no adverse effects were observed in this study. However, as only a very limited number of animals was included in this study (one per sex in Part A and one per sex in Part B) and only a limited number of parameters was investigated, the RMS considers this study as unacceptable and no conclusion on a NOAEL is drawn by the RMS.

In the DAR a NOAEL of 1000 mg/kg bw/day has been derived based on this study. In the evaluation provided in the RAR (2015) it was only stated that "*at least "part B" in which a limit dose of 1000 mg glyphosate/kg bw was administered daily for two weeks to dogs was also considered acceptable whereas part A was difficult to interpret due to a very unusual design with increasing doses*."

B.6.3.1.6. Five day oral toxicity study in dogs (isopropylamine salt of glyphosate)

Data point:	CA 5.3.1/008
Report author	[redacted]
Report year	1982
Report title	Range Finding Study of MON 0139 and Isopropylamine Administered Orally to Dogs
Report No	[redacted] 2155
Document No	Not reported
Guidelines followed in study	None specified
Deviations from current test guideline:	This study was a dose range-finding study for which there are no guidelines available. The examinations were confined to the occurrence of clinical signs, determination of body weight and food consumption and to a gross pathological examination at necropsy.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	GLP was not compulsory when study was conducted/ Study appears in compliance with FDA GLP requirements 21 CFR Part 58./Study conducted at [redacted]
Acceptability/Reliability:	**Conclusion GRG**: Supportive, category 2a **Conclusion AGG**: The RMS considers this study with the isopropylamine salt of glyphosate as acceptable but with restrictions (reliable with restrictions) (as a dose range finding study) as this study is designed as a non-guideline dose-range finding study with few animals and limited parameters investigated.

Executive Summary

The purposes of this study were: 1) to determine the potential toxicity of MON 0139 (the isopropylamine salt of glyphosate) and isopropylamine (IPA) alone administered orally to dogs, and 2) to provide information to assist in determination of dose levels for a subsequent 6-week study of both materials administered to dogs. Five male and four female purebred beagle dogs were administered MON 0139 and/or isopropylamine (IPA) by gavage or

gelatin capsules. Dosages of MON 0139 ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days. No animals died, and all dogs were killed and necropsied after varying observation periods. Mild weight loss and reduced food consumption occurred on and shortly after treatment days with MON 0139, however, both effects were reversible. Diarrhoea was seen at all dose levels of MON 0139 and emesis at all but the lowest dose. Two dose levels of IPA were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. IPA treatment resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs of the higher dose level dogs. Mucosal erosions of the stomach and oesophagus were observed in the lower level dog.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 0139 (Isopropylamine salt of glyphosate); Isopropylamine
Description:	Clear Liquid (MON 0139); Clear, slightly amber liquid (Isopropylamine)
Lot/Batch#:	LURT-12011 (MON 0139); Luling 2-81 (Isopropylamine)
Purity:	62.49 % (MON 0139); 99.7 % (Isopropylamine)
Stability of test compound:	Not evaluated

2. Vehicle and/or positive control: No controls utilised.

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	6–7 months
Sex:	Male and female
Weight at dosing:	♂ 7.3–10.0 kg; ♀ 7.5–9.4 kg when put on test
Acclimation period:	At least one month
Diet/Food:	Ralston-Purina Certified Dog Chow® #5006, 400 g/day
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages
Environmental conditions:	Temperature: 20 – 24 °C (68 – 75 °F) Humidity: 45 – 65 % Air changes: Not indicated 12 hours light/dark cycle

B: Study design and methods

In life dates: 1981-03-24 to 1981-05-18

Animal assignment and treatment:
Five male and four female purebred beagle dogs were administered MON 0139 or Isopropylamine (IPA) neat by gavage or gelatin capsules. Individual animals received dosages of MON 0139 as a single dose and/or daily doses for 5 days (See table below).

Due to the preliminary nature of this range-finding study, there were several additions and deviations to the study protocol.

The test materials were given neat by gavage or in gelatin capsules at dosages of 312.5, 625, 1250 or 2500 mg/kg bw/day (MON 0139), or 72 or 19.43 mg/kg bw/day (IPA). Analysis of the physical state, chemical content or stability of the test material was not required by the protocol. All but one animal were assigned to groups of two animals (1 male, 1 female). The study protocol indicated that the test material would be administered by gavage and the study was started in that manner. However, when the volume being given was reduced to approximately 7 mL, the form of delivery was changed to the less traumatic method of gelatin capsule administration. With gavage, dosing of the neat test substance was followed with administration of an equivalent amount of water. Blue food colouring was added to capsules in some cases to help ascertain the presence of test material in vomitus. Three dogs were given isopropylamine alone to determine the level of toxicity of this test substance alone.

Single dose trials:
The first pair of dogs received single doses of 2500 mg/kg bw of MON 0139 by gavage using Tygon tubing. To determine the effect of food in the stomach, the male dog had not been fed that day, while the female dog had food available before and after dosing. On the third day following dosing, in an attempt to administer a dose that would not produce emesis, the same two dogs were re-weighed and given 1250 mg/kg bw of MON 0139. Both dogs had been fed before dosing to determine if the presence of food in the stomach might mitigate emesis. After 4 days, this pair of dogs were again dosed with 1250 mg/kg bw of MON 0139. This time, however, both dogs were fasted before dosing and offered food immediately afterwards. In a final trial with this pair of dogs the dosage was reduced to 625 mg/kg bw with fasting prior to and feed immediately after dosing. These dogs were then observed for 3 weeks, sacrificed and necropsied.

Repeated dosing trials:
As a result of the single dose trials, it was decided to administer doses of 625 mg/kg bw/day for 5 days in gelatin capsules to a new pair of dogs (1 male, 1 female). These dogs were fasted before dosing and fed after dosing. Dose volumes were adjusted for body weight changes each day of treatment. These dogs were sacrificed in good condition after 3 weeks and necropsied.

A third pair of dogs were dosed at 625 mg/kg bw/day of MON 0139 for 5 days and a fourth pair was dosed at 312.5 mg/kg bw/day for 5 days. Several drops of blue food colouring were added to each capsule each day to help determine the presence of test material in the vomitus. These dogs were also fasted prior to and fed after dosing. The dogs treated at a dose level of 312.5 mg/kg bw/day were observed for nearly 2 weeks after treatment and the dogs treated at a dose level of 625 mg/kg bw/day were observed for 1 week.

At the end of the observation period for the two dogs dosed with MON 0139 at 312.5 mg/kg bw/day, it was decided to administer to these dogs the approximately equimolar amount of isopropylamine (IPA) alone contained in the 312.5 mg/kg bw/day dosage of MON 0139. The dosage of IPA given was determined to be 72 mg/kg bw as a single dose given neat in gelatin capsules. Both dogs were fasted before dosing and food was made available after dosing. Within 5 minutes both dogs vomited copious amounts of mucus and frank blood. This continued for 30 minutes, after which time both animals were sacrificed for humane reasons.

In a final trial, one new male dog was given daily doses of IPA diluted to 62.49 % in water via gelatin capsule for five days. The dosage (19.43 mg/kg bw/day) was calculated to be equivalent to the amount of IPA that would be present in a dosage of 75 mg/kg bw/day of MON 0139. The dog was fasted prior to and fed after dosing. The dog was sacrificed and necropsied the day after the last dose.

Table 6.3.1-1: Range Finding Study of MON 0139 and Isopropylamine Administered Orally to Dogs ([redacted] 1982): Study design

Dose	Number of doses	Dogs	Observation period
Mon 0139			
2500 mg/kg bw	1	1♂[a], 1♀[a]	3 days
1250 mg/kg bw	1	1♂[a], 1♀[a]	4 days
1250 mg/kg bw	1	1♂[a], 1♀[a]	2 days
625 mg/kg bw	1	1♂[a], 1♀[a]	3 weeks
625 mg/kg bw/day	5	1♂[b], 1♀[b]	3 weeks
625 mg/kg bw/day	5	1♂[c], 1♀[c]	2 weeks
312.5 mg/kg bw/day	5	1♂[d], 1♀[d]	3 weeks

Table 6.3.1-1: Range Finding Study of MON 0139 and Isopropylamine Administered Orally to Dogs ([redacted] 1982): Study design

Dose	Number of doses	Dogs	Observation period
Mon 0139			
Isopropylamine			
72 mg/kg bw	1	1♂[d], 1♀[d]	35 minutes (dogs sacrificed in extremis)
19.43 mg/kg bw/day	5	1♂[e]	5 days

Superscripts a, b, c, d indicate the same individual animals. For example, animals with superscript "a" received several different dose levels of the substance during the study.

Mortality
Each animal was observed a minimum of twice daily.

Clinical observations
Each animal was observed a minimum of twice daily.

Body weight
Animals were weighed on each day of dosing and weekly thereafter.

Food consumption and utilisation
Individual food consumption was estimated usually daily.

Ophthalmoscopic examination
Ophthalmoscopy was not performed.

Haematology and clinical chemistry
Haematological and clinical chemistry evaluations were not performed.

Urinalysis/Faecal analysis
Urine and faecal analyses were not performed.

Sacrifice and pathology
All animals were killed at the termination of their portion of the study and were necropsied. Survivors were fasted overnight prior to sacrifice in order to facilitate gross observation of intestinal and gastric organs. No organs were weighed. Only one kidney and two lymph nodes were retained but not examined microscopically.

Statistics
Statistical evaluation of the data was not performed since there were no more than two animals/dose/sex in a group.

II. RESULTS AND DISCUSSION

A. MORTALITY

Animals receiving MON 0139 at all doses survived to the end of the observation period. 2 dogs that received IPA alone, 72 mg/kg, (Equimolar to 312.5 mg/kg MON 0139) were killed in extremis within 30 minutes of dosing.

B. CLINICAL OBSERVATIONS

The first pair of dogs that received single doses of 2500 mg/kg bw of MON 0139 vomited approximately 30 minutes post dosing; the male's vomitus was clear with mucus present; the female's contained moist food and mucus. The same two dogs given 1250 mg/kg bw of MON 0139 vomited, the female approximately 50 minutes post dosing and the male 2 hours post dosing. There were no other indications of toxicity for the following four days. This pair was again dosed with 1250 mg/kg bw of MON 0139 which led to vomiting about one hour post dosing. The female vomited and had diarrhoea the next day and the male did likewise two days after dosing. This pair of dogs, after receiving 625 mg/kg bw did not vomit but the male had loose stools later in the day and on the following day. The male had one occurrence of diarrhoea during the final week before sacrifice.

For the second pair of dogs dosed with 625 mg/kg bw/day of Mon 0139 for 5 days, both dogs vomited material, presumably food, test material and gelatin. This usually occurred within 30 minutes after dosing. The male vomited each day of dosing, the female on the second and third days only. The female also had diarrhoea the last three days of dosing and the fourth day's stool had a greenish, mucoid appearance. The female had diarrhoea twice more during the observation period.

The third pair of dogs that received 625 mg/kg bw/day of MON-0139 for 5 days, also showed emesis. Both dogs vomited bluish material which usually occurred within 30 minutes after dosing. Diarrhoea occurred in both dogs on one day the week following treatment. The bluish vomitus colour and green stools observed for these animals was considered due to the tracking food colour added to the dose.

The fourth pair of dogs dosed at 312.5 mg/kg bw/day of MON-0139 for 5 days did not vomit at any time. The female at this dose level had diarrhoea on the second and third day of dosing and both dogs had green stools, probably due to the food colouring.

Both dogs dosed with IPA at 72 mg/kg bw as a single dose vomited copious amounts of mucus and frank blood within 5 minutes of dosing. This continued for 30 minutes, after which time it was deemed desirable to humanely sacrifice both animals.

The final male dog given daily doses of IPA at 19.43 mg/kg bw/day (diluted to 62.49 % in water) for 5 days vomited each of the first three days of dosing, with blood in the vomitus on days 2 and 3. Loose stool was the only observation on days 4 and 5 of dosing.

C. BODY WEIGHT

For dogs dosed for 5 days at 625 mg/kg bw/day of MON 0139 and observed for 3 weeks, the male's weight was steady for two weeks then increased by 1 kg during the final week of observation. The female also gained weight, 1.2 kg during the final week. The second pair of dogs dosed for 5 days at 625 mg/kg bw/day of MON 0139, lost a small amount of weight, the male 0.2 kg and the female 0.3 kg by the fifth day. The male regained lost weight within three days following the last day of treatment. However, the female had several episodes of diarrhoea and did not regain the lost weight for 2 weeks.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

For the first pair of dogs that received single doses of 2500 mg/kg bw of MON 0139, the male dog consumed all his food the 2 days following dosing; the female ate approximately one-fourth to one-third of her ration the following day and all of it the second day after dosing.

The pair of dogs given 625 mg/kg bw/day of MON 0139 for 5 days and observed for 3 weeks ate an average of one-half of their food during the 5 days of treatment. Both dogs' appetites improved; they ate an average of nearly three-fourths of their rations the second week and all of them the third week. The other pair of dogs given 625 mg/kg bw/day of MON-0139 for 5 days and observed for 2 weeks ate approximately 60 % of the food offered during the 5 days of treatment. The following week, the dogs consumed nearly all of their rations.

Food consumption for the pair of dogs dosed at 312.5 mg/kg bw/day of MON 0139 for 5 days averaged nearly 80 % of the food offered. The following week, the dogs consumed nearly all of their rations.

The final male dog on this study given daily doses of 19.43 mg/kg bw/day of IPA (diluted to 62.49 % in water) for 5 days daily ate all of the food offered to him.

E. OPHTHALMOSCOPIC EXAMINATION

Not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Not performed.

Blood clinical chemistry

Not performed.

G. URINALYSIS/FAECAL ANALYSIS

Not performed.

H. NECROPSY

Organ weights

Organ weights were not determined.

Gross pathology

For the pair of dogs dosed once with 625 mg/kg bw of MON 0139 and observed for 3 weeks, neither of the animals had any gross lesions at necropsy.

For dogs dosed at 625 mg/kg bw/day for 5 days and observed for 3 weeks, gross necropsy revealed no lesions for either dog.

For dogs dosed at 625 mg/kg bw/day for 5 days and observed for 2 weeks, gross necropsy revealed no lesions in the female animal. The male dog had dark areas, approximately 0.5 to 1 cm in diameter, in both submandibular lymph nodes.

No gross lesions were reported for the pair of dogs dosed at 312.5 mg/kg bw/day of MON 0139 for 5 days.

The pair of dogs dosed once with 72 mg/kg bw of IPA via capsule and humanely sacrificed after 30 minutes showed severe oedema, haemorrhage, and necrosis of the rugae of the stomach at necropsy.

The final male dog on this study given daily doses of 19.43 mg/kg bw/day of IPA (diluted to 62.49 % in water) for 5 days showed diffuse, eroded areas in the mucosal layer of the cardiac portion of the stomach, and two focal erosions were seen in the oesophageal mucosa at gross necropsy the day following the last treatment.

Histopathology

Not performed.

III. CONCLUSIONS

Five male and four female purebred beagle dogs were administered MON 0139 and/or isopropylamine (IPA) by gavage or gelatin capsules. Dosages of MON 0139 ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days. No animals died, and all dogs were killed and necropsied after varying observation periods. Mild weight loss and reduced food consumption occurred on and shortly after treatment days with MON 0139, however, both effects were reversible. Diarrhoea was seen at all dose levels of MON 0139 and emesis at all but the lowest dose. Two dose levels of IPA were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. IPA treatment resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs of the higher dose level dogs. Mucosal erosions of the stomach and oesophagus were observed in the lower level dog. A no-effect level was not established for MON 0139 in this study; however, a dosage level of 312.5 mg/kg bw/day was determined that was non-emetic. A preliminary evaluation of the toxicity of IPA was accomplished.

Assessment and conclusion by applicant:

This study was a dose range-finding study conducted in a small number of dogs performed primarily to determine an oral dose of MON 0139 that could be tolerated by dogs to provide data for setting doses for subsequent repeated-dose studies in this species. In addition, preliminary comparative information was obtained on the toxicity of MON 0139 (the isopropylamine salt of glyphosate) and of isopropylamine (IPA) alone administered orally to dogs.

Individual dogs of each sex were administered several dose levels of MON 0139 and/or isopropylamine (IPA) once or daily for 5 days. Dosing was by gavage or gelatin capsules with varying regimens of fasting and feeding before and after dosing to try to control emesis. Dosages of MON 0139 ranged from 312.5 to 2500 mg/kg bw/day in single doses or daily doses for 5 days.

No animals receiving MON 0139 died. Mild body weight loss and reduced food consumption occurred on and shortly after treatment days with MON 0139, however, both effects were reversible. Diarrhoea was seen at all

dose levels of MON 0139 and emesis at all but the lowest dose. Two dose levels of IPA were given: 72 mg/kg bw as a single treatment to a pair of dogs, and 19.43 mg/kg bw/day for five days to a single dog. Emesis, bloody emesis and loose stools were observed. IPA treatment resulted in severe oedema, haemorrhage, and necrosis of the rugae in the stomachs of the higher dose level dogs (these dogs were sacrificed *in extremis* on humane grounds 30 minutes after dosing). Mucosal erosions of the stomach and oesophagus were observed in the lower IPA dose level dog.

A NOAEL was not established for MON 0139 in this study; however, a dosage level of 312.5 mg/kg bw/day was determined as non-emetic.

Assessment and conclusion by RMS:
The RMS agrees with the assessment by the applicant. No further comments.

In the previous assessment in the RAR (2015), the following was concluded by the RMS DE:
"A study by [redacted] (1982, TOX9552349) in dogs does also not completely comply to modern standards and may not be used for risk assessment of glyphosate but is of interest because higher toxicity of a formulation as compared to the active ingredient was elucidated. Strong gastrointestinal irritation became apparent when the isopropylamine (IPA) salt of glyphosate (MON 0139) was administered to Beagle dogs. Vomiting and diarrhoea together with a reduction in body weight gain were observed at a dose level of 625 mg/kg bw and above. A dose of 312.5 mg/kg bw given for five days still caused a slight decrease in food consumption. Toxicity of isopropylamine alone was also tested in this experiment revealing strong irritation of gastric and esophageal mucosa at a much lower dose of 72 mg/kg bw upon single and repeated administration suggesting that these effects were most probably not or only partly attributable to glyphosate itself."

B.6.3.1.7. *In vitro* and *in vivo* 28-day study on effect of glyphosate on renal proximal tubule cells

Data point:	CA 5.3.1/009
Report author	Gao, H. *et al.*
Report year	2019
Report title	Activation of the N-methyl-D-aspartate receptor is involved in glyphosate-induced renal proximal tubule cell apoptosis
Document No	doi.org/10.1002/jat.3795 E-ISSN: 1099-1263
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG**: Yes/Reliable with restrictions **Conclusino AGG**: The *in vivo* part of the study is considered as reliable with restrictions, as this study was an investigative study which only included observation of body weight, liver and kidney weight, kidney histology and several other kidney parameters. The *in vitro* part is also considered as reliable with restrictions.

Full summary of the study according to OECD format
Glyphosate-based herbicides have been used worldwide for decades and have been suggested to induce nephrotoxicity, but the underlying mechanism is not yet clear. In this study, a human renal proximal tubule cell line (HK-2) was treated with glyphosate for 24 hours at concentrations of 0, 20, 40 and 60 μM. A cell culture model and an animal exposure model were used to investigate the influences of glyphosate on renal proximal tubule cells and the potential role of the N-methyl-D-aspartate receptor

(NMDAR) in response to glyphosate exposure.

Materials and methods

Chemicals – Glyphosate (N-phosphonomethyl) glycine [96%]) and glyphosate monoisopropylamine salt (purity 96 %), as a 40 % w/w solution in water were purchased from Millipore Sigma, St. Louis, USA.

Cell culture and treatment - The human renal proximal tubular epithelial cell line HK-2 was obtained from the American Type Culture Collection. The cells were cultured in RPMI 1640 medium supplemented with 10 % foetal bovine serum and 1 % penicillin/streptomycin in a humidified incubator with 5 % CO_2 at 37 °C. Glyphosate monoisopropylamine salt solution (40 % w/w in water) was used to prepare a glyphosate stock solution (200 mM), which was then was diluted in complete medium to the final concentrations. HK-2 cells were exposed to glyphosate at 0, 20, 40, 50, 60, 70, 80, 90 or 100 µM for 24 hours. For intervention experiments, HK-2 cells were pretreated with MK-801 at 100 µM for 12 hours, BAPTA-AM at 2 µM for 12 hours or NAC at 2 mM for 12 hours followed by treatment with glyphosate at 40 µM.

Cytotoxicity assays - Cell viability and death were evaluated by Cell Counting Kit-8 (CCK-8) and lactate dehydrogenase (LDH) cytotoxicity assay kit following the manufacturer's instructions. To determine cell viability, HK-2 cells were seeded into a 96-well plate (1×10^4 cells/mL) and then exposed to various concentrations of glyphosate for 24 hours. Cell-free medium and cells treated with water served as the blank and solvent controls, respectively. A mixture of 10 µL of CCK-8 solution and 90 µL of culture medium was added to each well and then incubated at 37 °C for 2 hours. Optical density at 450 nm was measured with a Synergy™ HT Microplate Reader. During the detection of glyphosate cytotoxicity, cell supernatants from each well were collected and then incubated with the LDH assay solutions for 30 minutes at 25 °C. Cell-free medium and cells treated with water served as the blank and solvent controls, respectively. Cells treated with lysis buffer and lysis buffer alone served as the positive and positive blank controls, respectively. Optical density at 490 nm was measured using a Synergy™ HT Microplate Reader.

Annexin V-fluorescein isothiocyanate/propidium iodide apoptosis assay - Cell apoptosis was measured using an Annexin V-fluorescein isothiocyanate apoptosis detection kit. Glyphosate- or vehicle-treated HK-2 cells were harvested and suspended in binding buffer. Annexin V-fluorescein isothiocyanate (5 µL) was added to the cells, followed by the addition of propidium iodide (PI, 5 µL). Water served as the solvent control, and apoptosis inducer A (Apopida) in the Apoptosis Inducer Kit served as the positive control. Cells without Annexin V and PI were used as negative controls. Subsequently, the cells were labeled for 15 minutes at 37 °C. The fluorescence intensity of Annexin V and PI was recorded using a flow cytometer. Data from 10,000 events per sample were analysed using FlowJo™ software.

Cellular reactive oxygen species measurement - Intracellular ROS production was measured by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) using flow cytometry. HK-2 cells were incubated with 100 nM DCFH-DA for 30 minutes at 37 °C. Cells were then harvested and resuspended in basal medium. Water and hydrogen peroxide were used as the solvent and positive controls, respectively. Cells without the DCFH-DA probe were used as a negative control. The fluorescence intensity of 3×10^4 cells per sample was acquired using a flow cytometer at an excitation wavelength of 488 nm and an emission wavelength of 530 nm. Data were analysed using FlowJo™ software.

Detection of intracellular Ca^{2+} levels -

Intracellular Ca^{2+} concentration was analysed using a Fluo-4/AM fluorescent probe. The cells were incubated with 2 µM Fluo-4/AM in Hanks' balanced salt solution at 37 °C for 30 minutes and then suspended in Hanks' balanced salt solution and incubated at 37 °C for an additional 20 minutes. H_2O and ionomycin (5 µM) were used as the solvent and positive controls, respectively. Cells without Fluo-4/AM probe were used as a negative control. Cell analysis was performed on a flow cytometer at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The $[Ca^{2+}]_i$ value is represented by the mean fluorescent intensity.

Western blotting - Untreated and treated cells were washed three times with ice-cold PBS and then lysed by RIPA lysis buffer containing 1 % protease inhibitors. The cell lysate was centrifuged and the supernatant was collected. The total cellular protein concentration was determined using a BCA assay kit. Equal amounts of total proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis through a 12 % gel and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5 % nonfat milk in PBS + Tween 20 (pH 7.5) at room temperature for 2 hours. Subsequently, the membranes were washed with PBS + Tween 20 and incubated with primary antibodies against human NMDAR1, Bcl-2, Bax, Bcl-xl, Bad, cleaved caspase-3 or β-tubulin overnight at 4 °C. The membranes were then incubated with antirabbit horseradish peroxidase-conjugated IgG antibodies for 2 hours at room temperature. The protein bands were visualized using an ECL detection kit. β- Tubulin served as an internal loading control.

Animals and treatment - Adult male ICR mice (aged 8 weeks) were obtained from the Shanghai Jiesijie Laboratory Animal Company (Shanghai, China) with license number SCXK (Hu)2013-0006. The mice were provided food and water ad libitum and maintained in a controlled environment at a temperature of 24 ± 1 °C, humidity of 45 ± 5 % and a 12-hour light/dark cycle. The animals were randomly assigned to the control group or the glyphosate exposure group (6 mice per group). The mice in the glyphosate exposure group received 400 mg/kg bw/day glyphosate via oral gavage once per day for a period of 28 days. For administration, glyphosate was diluted in an aqueous suspension and given to the mice once per day in a volume of 0.1 mL/10 g of body weight. The mice in the control group received distilled water. Body weight and food intake were measured daily. Urine was collected once a week using metabolic cages. At the end of the exposure period, blood was collected from the orbital venous plexus under anesthesia to prepare serum. Then, the animals were sacrificed and kidneys excised and washed with saline. The kidney samples were fixed by immersion in a 4 % paraformaldehyde solution for 24 hours at 4 °C for histology, immunohistochemistry and TUNEL examinations. The remaining samples were snap-frozen in liquid nitrogen and maintained at −80 °C for subsequent laboratory analysis.

Urine biomarker measurement - Urine and serum were frozen and stored at −80 °C immediately after collection and centrifugation. Urine creatinine, uric acid, urea and serum creatinine levels were determined using the respective assay kits. Urine β2-microglobulin and albumin levels were measured using enzyme-linked immunosorbent assay kits.

Superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde measurements - At the end of glyphosate treatment, the culture supernatants of treated and untreated HK-2 cells for the *in vitro* experiment or renal tissue for the *in vivo* experiment were freshly collected on ice for measurements of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) activities using the corresponding assay kits. SOD activity was determined according to the xanthine oxidase method, CAT activity was determined using the colorimetric method, GSH-Px activity was determined according to the ultraviolet spectrophotometric method, and MDA levels were determined using the thiobarbituric acid method. All experiments were performed following the manufacturer's instructions.

Kidney histopathology – Kidney tissue fixed for histopathological examination was dehydrated and embedded in paraffin. Tissue sections of 5 μm were mounted on poly-L-lysine-coated slides, deparaffinised with xylene, and stained with hematoxylin and eosin. A trained pathologist blinded to the treatments evaluated the tissue slides using an optical microscope.

Immunohistochemistry - Because the chemical structure of glyphosate and that of its metabolite AMPA are similar to glycine and glutamate, which are agonists of the N-methyl-D-aspartate receptor (NMDAR), the potential role of the NMDAR pathway in mediating the proapoptotic effect of glyphosate on proximal tubule cells was investigated. The kidney samples fixed for immunohistochemical examination were dehydrated, embedded in paraffin and cut in 5-μm thick slices. The slides were then deparaffinised and incubated with rabbit monoclonal anti-NMDAR1 primary antibodies at 4 °C overnight, washed in PBS and incubated with goat antirabbit IgG-horseradish peroxidase secondary antibodies for 50 minutes at room temperature. Next, 50 mL of 3,3'-diaminobenzidine was added to each kidney section, which was stained for 5 minutes. After the slides were washed, they were counterstained with hematoxylin for 3 minutes. The slides were then mounted and examined under a microscope. NR1 expression was quantified using Image-Pro Plus 6.0 software. Two negative controls were used: PBS treatment in place of primary antibody and an isotype-matched nonspecific antibody (normal rabbit IgG). Brain tissue from mice was used as a positive control.

TUNEL assay - Apoptotic cells were detected with a TUNEL assay kit according to the manufacturer's instructions. 4'-6-diamidino-2-phenylindole was used for counterstaining. Kidney tissue samples fixed for TUNEL

examination were dehydrated, embedded in paraffin and cut in tissue sections of 5 µm. The sections were then deparaffinised, rehydrated and treated with Protease K, after which they were incubated with the TUNEL reaction mixture in a humidified chamber at 37 °C for 2 hours. The sections were washed with PBS (pH 7.4) to terminate the reaction and then treated with 4'-6-diamidino-2-phenylindole. Sections treated with DNase before TUNEL examination served as a positive control, and sections without terminal deoxynucleotidyl transferase were used as a negative control. From each section, 5 randomly selected fields (200 × magnification) were photographed with a fluorescence microscope. The number of TUNEL-positive cells in each field was counted using Image-Pro Plus 6.0 software and divided by the field area. The average apoptotic cell density of the 5 fields was then obtained for each group.

Results

Cytotoxicity and apoptosis in HK-2 cells – Glyphosate reduced statistically significantly the number of viable cells at ≥ 40 µM and increased LDH release from 40 µM on. In addition, the cytotoxic effect of glyphosate on HK-2 cells was found to be dose- and time-dependent upon prolongation of the exposure times. Cell viability of both the 40 and 60 µM exposure groups was reduced but still greater than 50 % after 24 hours of exposure. Therefore, subsequent experiments were conducted with 0, 20, 40 and 60 µM with 24-hour exposure periods. In comparison with the control group, the glyphosate exposure groups showed increased percentages of apoptotic cells (Annexin V+, PI+/−), with significant differences occurring at concentrations greater than 40 µM. Furthermore, the expression of apoptosis-related proteins was investigated using Western blotting where in comparison to the control group glyphosate exhibited upregulated proapoptotic proteins (Bax and Bad) at 20 and 40 µM while downregulated antiapoptotic proteins (Bcl-2 and Bcl-xl) were evident at 40 and 60 µM. Cleaved caspase-3 levels were significantly upregulated at 40 and 60 µM.

Oxidative stress, NMDAR1 expression and calcium influx in HK-2 cells - In comparison with the control group, glyphosate increased statistically significantly cellular ROS levels at 40 and 60 µM. SOD, CAT and GSH-Px levels were statistically significantly reduced whereas there was a statistically significant increase in the MDA level. Glyphosate exposure increased NMDAR1 expression in a dose-dependent manner. Because NMDAR is involved in calcium influx and calcium homeostasis, $[Ca^{2+}]_i$ levels were determined after glyphosate exposure using flow cytometry-based measurement with the Ca^{2+}- sensitive probe Fluo-4/AM. It was found that glyphosate exposure increased $[Ca^{2+}]_i$ levels.

Effects after pretreatment with MK-801 - To examine the role of NMDAR in glyphosate-induced calcium influx and apoptosis, HK-2 cells were pretreated with the NMDAR inhibitor MK-801 at 100 µM for 12 hours followed by treatment with 40 µM glyphosate for 24 hours. MK-801 attenuated the upregulation of $[Ca^{2+}]_i$ in the HK-2 cells after 24 hours of glyphosate treatment. Inhibition of NMDAR was found to attenuate ROS increase in these cells and significantly decrease glyphosate-induced apoptosis.

Effects after pretreatment with BAPTA-AM and N-acetylcysteine - To examine the relationship between glyphosate-induced calcium influx and apoptosis, HK-2 cells were pretreated with 2 µM of an intracellular calcium chelator (BAPTA-AM) for 12 hours to decrease $[Ca^{2+}]_i$. Cells pretreated with BAPTA-AM and exposed to 40 µM glyphosate had lower $[Ca^{2+}]_i$ levels than cells exposed to glyphosate alone. BAPTA-AM pretreatment also significantly decreased glyphosate-induced apoptosis and ROS. To determine the role of ROS in glyphosate-induced apoptosis of HK-2 cells, cells were pretreated with 2 mM of a ROS scavenger (N-acetyl cysteine, NAC) for 12 hours before glyphosate treatment. NAC pretreatment was found to reduce glyphosate-induced ROS levels and apoptosis.

NMDAR1 expression and kidney damage - Glyphosate was administered orally to ICR mice for 28 days at a daily dose of 400 mg/kg bw to investigate its effects on the kidney *in vivo*. No statistically significant difference was found in body weight gain and relative liver and kidney weight between the control and the glyphosate exposure groups. Histopathological examination of the kidney identified exfoliation of renal tubular cells. The TUNEL assay confirmed the increase in renal tubular cell apoptosis in mice

exposed to glyphosate. No statistically significant changes were observed in urine creatinine, uric acid, urea nitrogen, serum creatinine and blood urea nitrogen levels. A transient increase in urine albumin was observed after 7 and 14 days of treatment and urinary β2- microglobulin levels were statistically significantly increased after 7, 21 and 28 days of treatment. In addition, statistically significant reductions in the levels of SOD, CAT and GSH-Px, and a statistically significant increase in MDA was observed in the kidneys of the glyphosate treated group. Besides, the average optical density for NMDAR1 was found to be increased in kidneys from glyphosate treated mice.

Discussion and conclusion

Glyphosate exposure was found to increase the production of ROS. In *in vitro* and *in vivo* it was shown that MDA levels increased and that the activities of the major endogenous antioxidant enzymes SOD, CAT and GSH-Px decreased as a result of glyphosate exposure which is indicative of the disturbance of the pro-oxidant/antioxidant balance. To understand how this balance is disturbed the role of the N-methyl-D-aspartate receptor (NMDAR) was investigated because the structure of glyphosate is similar to that of glycine and glutamate which are both agonists of this receptor. NMDAR has been reported to mediate some renal diseases, such as hyperhomocysteinemia-induced glomerulosclerosis, gentamicin nephrotoxicity and lipopolysaccharide-induced renal insufficiency. In this study, using the human renal tubular epithelial cell line HK-2, the increased expression of NMDAR1 protein was demonstrated upon glyphosate exposure. In the animal tests, a clear upregulation of NMDAR1 in renal tissue was observed in tubular epithelial cells using immunohistochemical staining. Accompanied by the increase in NMDAR1 upon glyphosate exposure, $[Ca^{2+}]_i$, oxidative stress markers and apoptosis were all increased. Blocking NMDAR not only ameliorated glyphosate-induced increases in $[Ca^{2+}]_i$ and ROS levels but also attenuated apoptosis. The increase in β2-microglobulin in the urine as observed in the *in vivo* study is indicative of an impairment of tubular reabsorption. From the results of this study it can be concluded that glyphosate could affect renal tubule epithelial cells via the NMDAR1/$[Ca^{2+}]_i$/ROS pathway both *in vitro* and *in vivo*. These findings provide a theoretical basis and reference data to assess the risk of glyphosate and to explore the ethiology of chronic kidney disease.

Assessment and conclusion by applicant:

In this study the effect of glyphosate on human proximal tubular epithelial cells was studied *in vitro* and on mouse kidney *in vivo*. Tubular epithelial cells (HK-2) *in vitro* were exposed to glyphosate at concentrations ranging from 20 to 100 µM whereas mice were orally treated with glyphosate at 400 mg/kg bw/day for 28 days. The endpoints investigated for the *in vitro* study were cell viability, apoptosis, oxidative stress, intracellular Ca^{2+}, expression of the N-methyl-D-aspartate (NMDA) receptor and expression of proteins involved in apoptosis. The endpoints explored in the *in vivo* study in mice were kidney pathology biomarkers, oxidative stress in kidney tissue, kidney histopathology, NMDA receptor immunochemistry and apoptosis in kidney tissue. Glyphosate was found to reduce cell viability, increase the incidence of apoptotic cells with an increase in the expression of apoptosis-related proteins, increase of oxidative stress in a concentration-related manner, increase of the expression of the NMDA receptor and increase Ca^{2+} influx. Kidney histopathology in mice treated with glyphosate at 400 mg/kg bw/day for 28 days revealed the exfoliation of renal tubular cells. It is postulated by the authors that glyphosate could affect renal tubule epithelial cells via the NMDAR1/$[Ca^{2+}]_i$/ROS pathway. The effects described in this study are not corroborated by regulatory 90–day repeated dose toxicity studies where no renal effects were seen in rats dosed up to more than 4000 mg/kg bw/day and mice dosed up to more than 7000 mg/kg bw/day.

Reliability criteria for *in vitro* toxicology studies made by the applicant

Publication: Gao *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		

Reliability criteria for *in vitro* toxicology studies made by the applicant

Publication: Gao *et al.*, 2019	Criteria met? Y/N/?	Comments
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	Y	Purity of 96 % as glyphosate monoisopropylamine salt. Source: Millipore Sigma, St. Louis, 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	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	Not reported	
Test concentrations in physiologically acceptable range (< 1 mM)	Y	Concentration range *in vitro* from 20 to 100 µM. Only one dose (400 mg/kg bw/day) was used in the oral toxicity study in mice.
Cytotoxicity tests reported	Y	
Biochemical methods described	Y?	Some could be better documented.
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	Y	*In vitro* but not *in vivo* (only one dose used).
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 biochemical methods are not sufficiently described, only one dose was used in the *in vivo* study and the pathology results from the *in vivo* study are not corroborated by regulatory 90–day repeated dose toxicity studies where no renal effects were seen in rats dosed up to more than 4000 mg/kg bw/day and mice dosed up to more than 7000 mg/kg bw/day.		

Assessment and conclusion by RMS:

The RMS agrees with the assessment by the notifier. In the *in vitro* part of the study, glyphosate (as monoisopropylamine salt solution (40 % w/w in water)) was found to reduce cell viability, to increase the incidence of apoptotic cells with an increase in the expression of apoptosis-related proteins, to increase of oxidative stress in a concentration-related manner, to increase of the expression of the NMDA receptor and to increase Ca2+ influx. In the *in vivo* part of the study, kidney histopathology revealed the exfoliation of renal tubular cells in the animals treated with glyphosate at 400 mg/kg bw/day during 28 days. Also, upregulation of apoptosis and NMDAR1 exposure in the proximal tubule epithelium and an imbalance of oxidant/antioxidant balance were observed. Further, a transient increase in urine albumin was observed after 7 and 14 days of treatment (1.8- to 2.0-fold increase compared with controls) and urinary β2-microglobulin levels were statistically significantly increased after 7, 21 and 28 days of treatment (1.7- to 3.5-fold increase compared with controls). Based on this mechanistical study, the authors postulated that glyphosate could affect renal tubule epithelial cells via the NMDAR1/[Ca2+]i/ROS pathway.

It should be noted that in the *in vitro* experiment glyphosate was administrated as a monoisopropylamine salt

solution (40% in water) and in *the vivo* experiment as glyphosate.

B.6.3.1.8. Five week oral exposure study in rats investigating liver oxidative damage

Data point:	CA 5.3.1/011
Report author	Tang, J. *et al.*
Report year	2017
Report title	Ion imbalance Is Involved in the Mechanisms of Liver Oxidative Damage in Rats Exposed to Glyphosate
Document No	doi.org/10.3389/fphys.2017.01083 ISSN: 1664-042X
Guidelines followed in study	None
Deviations from current test guideline	No
GLP/Officially recognised testing facilities	Non-GLP
Acceptability/Reliability:	**Conclusion GRG**: Yes/Reliable with restrictions **Conclusion AGG**: The study is considered as supportive as the purity of the test substance was not provided, only 8 instead of 10 animals were treated per dose group and only males were treated.

Full summary of the study according to OECD format

This study aimed to investigate the effects of glyphosate on rats' liver function and induction of pathological changes in ion levels and oxidative stress in hepatic tissue. Sprague-Dawley rats were treated orally with 0, 5, 50, or 500 mg/kg body weight of the glyphosate (GLP). After 5 weeks of treatment, blood and liver samples were analysed for biochemical and histomorphological parameters. The various mineral elements content in the organs of the rats were also measured. Significant decreases were shown in the weights of body, liver, kidney and spleen between the control and treatment groups. Changes also happened in the histomorphology of the liver and kidney tissue of GLP-treated rats. The GLP resulted in an elevated level of glutamic-oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and IL-1b in the serum. Besides, decreased total superoxide dismutase (T-SOD) activity and increased malondialdehyde (MDA) contents in the serum, liver, and kidney indicated the presence of oxidative stress. Moreover, increase of hydrogen peroxide (H_2O_2) level and catalase (CAT) activity in the serum and liver and decrease of glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in the kidney tissue further confirmed the occurrence of oxidative stress. The results of RT-PCR showed that the mRNA expressions of IL-1α, IL-1β, IL-6, MAPK3, NF-κB, SIRT1, TNF-α, Keap1, GPX2, and Caspase-3 were significantly increased in the GLP-treated groups compared to the control group. Furthermore, PPARα, DGAT, SREBP1c, and SCD1 mRNA expressions were also remarkably increased in the GLP-treated groups compared to the control group

Materials and methods

Chemicals - Glyphosate was obtained from Shanghai Ryon Biological Technology Co. Ltd (Shanghai, China). Purity was not reported.

Animals - Male Sprague-Dawley rats of 8 weeks old and weighing 180–220 g were purchased and were allowed to acclimate for at least one week prior to testing. All rats were housed in separate cages and had unrestricted access to food and water throughout the study.

Animal treatment and sample collection - Rats were randomly assigned to 4 groups (n = 8/group) and were orally administered glyphosate by gavage at 5, 50, and 500 mg/kg bw/day for 35 days. Glyphosate was orally administered at a volume of 0.5 ml/kg bw. Distilled water was used as the negative control. Twenty-four hours after the last gavage, rats were weighed and sacrificed. Blood samples were collected from the jugular vein and placed at 37°C for one hour before being centrifuged for biochemical assays. Liver, kidney, spleen, heart, lungs,

brain, adrenal glands, muscle and fat tissue were collected, rinsed with PBS, dried and weighed. A piece of liver and right kidney was used for morphometric analysis and another piece was used to prepare homogenates for the analysis of parameters of oxidative stress or frozen in liquid nitrogen for subsequent qualitative reverse transcription polymerase chain reaction (RT-PCR).

Histological preparation - Samples of liver and kidney were fixed in 4 % formaldehyde solution for 24 hours, dehydrated in alcohol, clarified with xylene, and embedded in paraffin. Paraffin blocks were sectioned into 5µm slices and stained with hematoxylin-eosin (HE) for microscopic examination.

Biochemical evaluation - Liver and kidney homogenate and serum were used for the assessment of liver function (serum GOT and GPT) and oxidative stress (total SOD, MDA, H_2O_2, CAT, GSH, and GSH-peroxidase or GSH-px). The activity of serum GOT and GPT was assayed according to the method that is normally applied in clinical biology. The analysis of total SOD activity was based on SOD-mediated inhibition of nitrite formation from hydroxyammonium in the presence of O^{2-} generators (xanthine/xanthine oxidase). Total SOD activity was expressed in U/mg protein. MDA was evaluated by the thiobarbituric acid reactive substances method (TBARS) and the results were expressed in nmol/mg protein. GSH-px activity was estimated by the determination of reduced GSH in the enzymatic reaction. GSH-px activity was expressed in U/mg protein. CAT activity was assayed by the method developed by Aebi, and calculated as nM H_2O_2 consumed/min/mg of protein. Protein concentrations in the supernatant were measured according to the Coomassie Brilliant Blue method.

Serum Cytokine Measures - Serum levels of IL-1β and IL-6 were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit.

Quantitative RT-PCR (qRT-PCR) analysis - Total RNA was extracted from tissue using the reagent box of Total RNA Kit, according to the manufacturer's instructions. The concentration of RNA was measured by spectrophotometry and the purity was ascertained by the A 260/A 280 ratio. Total RNA from each sample was reverse transcribed to cDNA with an Omniscript® Reverse Transcription kit with Oligo-dT primers according to the manufacturer's instructions and used for RT-PCR. The target fragments were quantified by real-time PCR with 100 ng of the cDNA template. Each sample was tested in duplicate. The gene expression data were normalized to β-actin expression. For each real-time PCR assay, the threshold cycle Ct was determined for each reaction. Ct values for each gene of interest were normalized to the housekeeping gene, β-actin and PCR amplification efficiencies were taken into account by amplifying various amounts of target cDNA for each reaction. The fold differences in mRNA expression of samples were relative to the internal control sample, which was included in all runs.

Ion Concentration - The concentrations of Al, Fe, Cu, Zn, and Mg in liver, kidney, spleen, lung, heart, muscle, brain, and fat tissue were determined by inductively coupled plasma optical emission spectrometry using nitric acid–perchloric acid–based wet digestion. Approximately 200 µL or 0.5 g of each sample was digested with nitric acid (75 %) and perchloric acid (25 %) in a microwave digester. The same part of the organ was used from the control and treated animals.

Statistical Analysis - The data were expressed as mean ± standard error of the mean (SEM) and were analysed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test, which was performed with GraphPad Prism software. Differences were considered to be statistically significant when $p < 0.05$.

Results

Body weight and body weight gain were statistically significantly decreased at 500 mg/kg bw/day and at 50 and 500 mg/kg bw/day, respectively. Absolute and relative organ weight was statistically significantly reduced for liver, spleen and kidney at 500 mg/kg bw/day.

Liver sections of rats exposed to glyphosate showed apoptosis of some hepatocytes, focal necrosis and mononuclear cell infiltration. At 5 mg/kg bw/day rats showed mild periportal expansion and apoptosis of some hepatocytes. At 50 and 500 mg/kg bw/day apoptosis of some hepatocytes and monocyte infiltration was observed in liver tissue. In the kidney, marked histological changes were observed, including proximal and distal tubular necrosis and glomerular toxicity. The histologic score of hepatic and renal damage was statistically significantly increased at all dose levels. Serum activity of GOT and

GPT was statistically significantly increased at 500 mg/kg bw/day.

Table 6.3.18-1 (added by RMS): Body weights and organ weights of rats treated with glyphosate for five weeks.

Parameter	Control	5 mg/kg bw/d	50 mg/kg bw/d	500 mg/kg bw/d
Body weight (g) - initial	298.60 ± 5.17	323.30 ± 4.94	313.40 ± 7.12	311.40 ± 8.87
Body weight (g) – wk 5	388.60 ± 7.08	404.00 ± 5.71	369.30 ± 12.57	**351.80 ± 7.74 ***
Body weight gain (%)	30.40 ± 3.18	23.42 ± 1.06	**17.38 ± 2.49 ** (-43%)**	**17.29 ± 5.41 * (-43%)**
Liver weight (g) – absolute	12.94 ± 0.45	12.98 ± 0.36	11.83 ± 0.74	**10.66 ± 0.44 ***
Liver weight (g) - relative	3.51 ± 0.07	3.30 ± 0.10	3.20 ± 0.21	**2.95 ± 0.09 ***
Spleen weight (g) – absolute	0.77 ± 0.04	0.71 ± 0.03	0.76 ± 0.06	**0.59 ± 0.04 * (-23%)**
Spleen weight (g) - relative	0.21 ± 0.01	0.18 ± 0.01	0.20 ± 0.01	**0.16 ± 0.01 * (-24%)**
Kidney weight (g) – absolute	1.19 ± 0.04	1.27 ± 0.003	1.18 ± 0.07	**1.00 ± 0.02 ***
Kidney weight (g) - relative	0.33 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	**0.29 ± 0.01 ***

* $P < 0.05$ and ** $P < 0.01$

Assessment of oxidative stress - In serum, total SOD activity was statistically significantly decreased at 500 mg/kg bw/day. MDA content was significantly increased at 50 mg/kg bw/day but not at 500 mg/kg bw/day and CAT activity was significantly increased at 500 mg/kg bw/day. In liver, total SOD activity was statistically significantly decreased and H_2O_2 levels increased at 500 mg/kg bw/day. In kidney, total SOD and GSH-px activities were significantly decreased at 50 and 500 mg/kg bw/day. A statistically significant decrease in GSH levels was observed at 50 mg/kg bw/day but not at 500 mg/kg bw/day.

Serum IL-1β and IL-6 levels - In serum, the level of IL-1β was statistically significantly increased at 500 mg/kg bw/day. There was no statistically significant change for IL-6.

Expression of genes related to inflammation in the Liver - Hepatic IL-1α and IL-1β mRNA expression were statistically significantly increased at all dose levels. IL-6, MAPK3, SIRT1, TNF-α, GPX2, and *Caspase*-3 mRNA expression was significantly increased at 50 and 500 mg/kg bw/day. NF-κB mRNA expression showed only a significant increase 50 mg/kg bw/day and *Keap*1 mRNA expression was only increased at 5 mg/kg bw/day.

Expression of genes related to lipid metabolism in the liver - PPARα, *SREBP1c*, and *SCD1* mRNA expression were significantly increased at 50 and 500 mg/kg bw/day and *DGAT* mRNA expression was significantly increased at 500 mg/kg bw/day.

Concentration of Al, Fe, Cu, Zn and Mg in tissues – The Al concentration was statistically significantly increased in liver at 50 and 500 mg/kg bw/day but significantly decreased in lung at 50 mg/kg bw/day and in muscle at 500 mg/kg bw/day. Fe was significantly increased at 5 mg/kg bw/day only in liver, at 50 mg/kg bw/day only in kidney and spleen and at 500 mg/kg bw/day in lung. Cu was significantly increased in brain and fat tissue at 500 mg/kg bw/day and Zn was only significantly increased in liver

at 50 and 500 mg/kg bw/day. Mg levels were only increased in brain tissue at 500 mg/kg bw/day.

Discussion and Conclusions

The results of this study showed that exposure to glyphosate for 35 days at doses up to 500 mg/kg bw/day led to a statistically significant reduction in body weight and body weight gain and in absolute and relative weight of liver, kidney and spleen. Histopathological examination of the tissues also revealed effects in liver and kidneys. Liver effects were corroborated by a significant increase in GOT and GPT. The results of this study showed that SOD activity was significantly decreased in serum, liver and kidney of rats treated with glyphosate when compared with the control group. MDA content was significantly increased in serum and kidney. CAT activity was also significantly elevated in serum and H_2O_2 levels were increased in liver tissue, suggesting oxidative stress. Taken together, the data demonstrated that glyphosate exposure could result in liver and kidney damage due to oxidative stress. The level of IL-1β was significantly increased at 500 mg/kg bw/day. Therefore, the relationship was investigated between oxidative stress and the transcription of genes related to inflammation. In this study, mRNA expression of *IL-1α, IL-1β, IL-6*, *MAPK3*, *NF-κB, SIRT1, TNF-α, Keap1, GPX2* and *Caspase-3* were all increased upon exposure to glyphosate. Genes related to lipid metabolism such as *PPARα, SREBP1c, DGAT*, and *SCD1* were significantly upregulated in rats exposed to glyphosate. The results from this study show that the liver toxicity induced by glyphosate is mediated by inflammation, oxidative stress and lipid related pathways. Tissue concentrations of Al, Fe and Zn were significantly increased in liver tissue of rats exposed to glyphosate. Concentrations of Fe were also increased in kidney, spleen, and lung tissue although not always in a dose-related manner. Al concentration was decreased in lung and muscle tissue whereas Cu concentrations were increased in brain and fat tissue and Mg in brain tissue of rats exposed to glyphosate. Combined, these results suggest that glyphosate exposure impaired the ion-balance of Al, Fe, Mg, Cu, and Zn.

Assessment and conclusion by applicant:
The objective of this study was to investigate the toxicity, oxidative stress and metal ion concentrations in tissues of rats after oral exposure to glyphosate for 35 days at doses up to 500 mg/kg bw/day. Oxidative stress was studied by the determination of markers of oxidative stress such as SOD, CAT, H_2O_2, MDA, GSH and GSH-px and the transcription of genes related to inflammation and lipid metabolism. Statistically significant effects were found on body weight, body weight gain, organ weight, serum indicators of liver toxicity and histopathology of the liver and the kidney. Significant changes were also reported on markers of oxidative stress and transcription of genes related to inflammation and lipid metabolism. Many of the effects reported were mild in nature and/or didn't show a clear dose-effect relationship. Also the effects on metal ion concentrations in organ tissues were not always consistent and often didn't show a dose-effect relationship. Moreover, the findings are not corroborated by the regulatory studies of similar test durations and dose ranges.

Reliability criteria for *in vivo* toxicology studies made by the applicant

Publication: Tang *et al*., 2017	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Number of animals per dose level lower than minimum required for 4–week testing
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	?	Not described in sufficient detail
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	Only source reported: Shanghai Ryon Biological

		Technology Co. Ltd., China.
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	Y	
Route and mode of administration described	Y	
Dose levels reported	Y	
Number of animals used per dose level reported	Y	
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	Sometimes inaccurate reporting of data in tables. Results not concordant with short term regulatory toxicology studies
Statistical methods described	Y	
Historical control data of the laboratory reported	N	
Dose-effect relationship reported	Y	
Overall assessment		
Reliable without restrictions		
Reliable with restrictions	Y	
Not reliable		
This publication is considered relevant but reliable with restrictions because the test material was not sufficiently characterised, the number of animals used for this study duration is not sufficient, the results were not always accurately reported and are not corroborated by regulatory toxicology studies of similar test durations and dose ranges.		

Assessment and conclusion by RMS:

The RMS considers this study as supportive only as the purity of the test substance was not provided, only 8 instead of 10 animals were treated per dose group and only males were treated.

The applicant has stated above that the results are not corroborated by regulatory toxicology studies of similar test durations and dose ranges. However, this is not completely agreed by the RMS as dose levels are covered by the current data package. Indeed a study with a duration of 35 days is not present in the dossier, however, both 28-day and 90-day studies are available. The major difference is, however, that the administration way used in this was gavage while the standard toxicity studies were feeding studies.

Based on the study, body weight was significantly decreased at the top dose, but as the changes were less than 10% compared with the corresponding controls these are not considered adverse. Body weight gain appeared to decrease with increasing dose. Despite no mean data is provided on the body weight gain during the five week treatment, based on body weight gain presented as a percentage, the reduced body weight gain at 50 and 500 mg/kg bw/day is considered adverse by the RMS. It should be noted that animals were not randomized based on their body weight and that the control group had a lower mean initial body weight than the animals in the treated groups. The increase in serum GPT and GOT at 500 mg/kg bw/day is not considered adverse as the increase was only slight. An absolute and relative decreased spleen weight of more than 10% compared with controls was observed at the top dose, which was considered adverse. Regarding liver and kidney, the decreased organ weights at the top dose were not considered adverse. Signs of oxidative stress, upregulation of liver inflammatory genes and upregulation of genes related to lipid metabolism were noted at 50 and 500 mg/kg bw/day. However, the effects were mostly slight and/or clinical relevance of these findings is lacking.

B.6.3.2. Oral 90-day studies

B.6.3.2.1. Oral 90-day toxicity study in rats – study 1

Data point	CA 5.3.2/001
Report author	[redacted]
Report year	1996 (Study Report)
Report title	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats
Report No	[redacted] P/1599
Document No	Not reported
Guidelines followed in study	No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)
Deviations from current test guideline (OECD 408, 2018)	No pre-dose ophthalmology, no reticulocyte count, T3, T4, TSH, less blood clinical chemistry parameters evaluated (no sodium, potassium, HDL, LDL, blood urea nitrogen and creatinine evaluated) thyroids, epididymis, prostrate, uterus, ovaries, thymus, spleen and pituitary gland not weighed, part of the tissues were stored and no histopathology was performed (eyes, Harderian gland, larynx, nasal cavity, mouth, prostrate, seminal vesicles, skin and voluntary muscle); vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point	CA 5.3.2/002
Report author	[redacted]
Report year	1996 (Appendix to Study Report)
Report title	First Revision to Glyphosate Acid: 90 Day Oral Feeding Study in Rats - Appendix
Report No	[redacted] P/1599
Document No	Not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	**Conclusion GRG**: Valid, category 2 **Conclusion AGG**: The study is considered reliable.

Executive Summary

In a sub-chronic toxicity study, groups of twelve male and twelve female Alpk:AP (now known as Alpk:APfSD) Wistar-derived rats were fed diets containing 0 (control), 1000, 5000 or 20000 ppm glyphosate acid for 90 consecutive days (equivalent to 0, 81.33, 413.5, or 1612 mg/kg bw/day in males and 0, 90.42, 446.9 or 1821 mg/kg bw/day in females).

Clinical observations, body weights, food consumption and blood biochemistry parameters were measured and at the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

Males fed 20000 ppm glyphosate acid showed small reductions in body weight gain, food consumption and food utilisation efficiency compared to controls. There were associated minor reductions in plasma urea, total protein and triglycerides. Increased levels of plasma ALT and ALP were seen in both male and female rats at the top dose and are considered to indicate an altered liver metabolism but there was no evidence of histological change in this organ.

Effects in rats fed 5000 ppm glyphosate acid were confined to marginal increases in plasma ALT levels in females and in both plasma ALT and ALP levels in males. The magnitude and isolated nature of these effects leads them to be considered of no biological significance.

Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry (ALP). The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change. Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats.

The RMS agrees with the NOAEL of 5000 ppm glyphosate acid (equivalent to 413.5 mg/kg bw/day for males and 446.9 mg/kg bw/day for females). At the LOAEL of 20000 ppm, body weight gain was reduced in males and alkaline phosphatase was increased in both males and females.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch#:	P15
Purity:	97.4%
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Alpk:APfSD (also known as Alpk:AP Wistar-derived)
Source:	[redacted]
Age:	36 – 38 days
Sex:	Male and female
Weight at dosing:	♂ 98 – 170 g; ♀ 96 – 140 g
Acclimation period:	Approximately 1 week
Diet/Food:	CTI diet, *ad libitum,* (except during collection of urine samples)
Water:	Filtered mains water, *ad libitum,* (except during collection of urine samples)
Housing:	4/cage, sexes separately in stainless steel cages 34.0 × 37.5 × 20 3 cm giving a floor area of 1275cm^2
Environmental conditions:	Temperature: 21 ± 2 °C (range 19 – 24 °C) Humidity: 36 – 60% Air changes: ≥15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1986-02-25 to 1986-05

Animal assignment and treatment:
The study was divided into six single-sex replicates (randomised blocks). Each replicate consisted of four cages, one per treatment group. The animals were randomly allocated to cages.
The study consisted of one control and three treatment groups each containing twelve male and twelve female rats.

Table 6.3.2-1: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Study design

Test group	Dietary concentration [ppm]	Males	Females
Control	0	12	12
Low	1000	12	12
Mid	5000	12	12
High	20000	12	12

The experimental diets were made in 60 kg batches by adding the appropriate amount of glyphosate acid to the diet using dry premixes.

Samples from all dietary levels (including controls) were taken from both batches prepared for the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in CT1 diet was determined by analysing samples from the low and high dose levels from the first batch of diet. The chemical stability of glyphosate acid in diet was determined at the highest and lowest dose levels at 1, 4, 6 and 10 weeks after preparation. Analysis was by high performance liquid chromatography (HPLC).

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before of the beginning of the treatment period and then once a week until the end of the study.

Body weight
The body weight of each animal was recorded immediately before feeding of the experimental diets commenced and then on the same day, where practicable, of each subsequent week until termination. The body weight determination was done on the same day on which the detailed clinical examination was performed.

Food consumption and utilisation
Food consumption was recorded continuously throughout the study for each cage of rats and calculated as a weekly mean (g food/rat/day) for each cage. The food utilisation value per cage was calculated as the body weight gained by the rats in the cage per 100 g of food eaten.

Ophthalmoscopic examination
The eyes of all animals from the control group and the 20000 ppm glyphosate acid dose level group were examined in the week prior to termination, using an indirect ophthalmoscope and a mydriate to dilate the pupil.

Haematology and clinical chemistry
At termination, all surviving rats were bled by cardiac puncture and the blood samples were collected both in tubes containing EDTA as anticoagulant and also in tubes containing 0.11 M trisodium citrate. These samples were submitted for haematological examination and the following parameters measured: haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), kaolin-cephalin times, thrombocytes, leucocytes, differential white cell count, red blood cell morphology, prothrombin time.

For clinical chemistry analysis blood samples were collected by tail vein bleeding at week 4 of the study and by cardiac puncture at termination (week 13). The blood was collected in lithium heparinised tubes and the following

parameters measured: glucose, urea, total protein, albumin, total cholesterol, triglycerides, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Urinalysis
Urine samples were collected over an 18 hour (approximately) period from all rats during week 13 (the week prior to termination). During urine collection, the rats were individually housed in metabolism cages and denied access to food and water. The following parameters were measured: volume, pH, specific gravity, proteins, glucose, ketones, and urobilinogen.

Sacrifice and pathology
On completion of the treatment period, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: adrenals, brain, heart, kidneys, liver, and testes. Paired organs were weight together.

Tissue samples were taken from the following organs and preserved in buffered formalin: all gross lesions, adrenals, aorta, bone marrow (femur), brain, caecum, colon, duodenum, epididymides, eyes (stored), Harderian gland (stored), heart, ileum, jejunum, kidneys, larynx (stored), liver, lungs, lymph nodes (cervical and mesenteric), mammary gland, nasal cavity (stored), mouth (stored), oesophagus, ovaries, pancreas, pituitary gland, prostrate (stored), rectum, salivary glands, seminal vesicles (stored), spinal cord, sciatic nerve, skin (stored), spleen, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus (with cervix) and voluntary muscle (stored).

Following fixation, all tissues from the control and 20000 ppm glyphosate acid groups (except those stored) were processed by standard methods, embedded in paraffin wax, sectioned at 5 µm, stained with haematoxylin and eosin and examined by light microscopy. Liver, kidney, adrenals, lungs and abnormal tissues from animals fed 1000 ppm or 5000 ppm glyphosate acid were also processed to blocks and were examined histologically.

Statistics
All data were evaluated using analysis of variance (body weight gain from start of study, final body weight, haematology, clinical chemistry – blood and urine, total food consumption and utilisation, organ weights) and covariance (organ weights on terminal body weights) for each specified parameter using the GLM procedure in SAS (1982). Unbiased estimates of the treatment group means were provided by the least square means (LSMEANS option in SAS). Each treatment group mean was compared to the control group mean using a two-sided Student's t-test, based on the error mean square in the appropriate analysis. Where male and female data were analysed together these comparisons were made for male and female means separately.

II. RESULTS AND DISCUSSION

DIETARY ANALYSIS
The mean concentrations determined in all of the diets prepared for this study were within 8% of the nominal concentration (92.8 to 101.7% of the nominal concentration). No glyphosate acid was found in the control diet. Satisfactory homogeneity of mixing was also demonstrated with values falling within ±3% of the overall mean concentration. Chemical stability of glyphosate acid in diet was determined by periodic re-analysis of the highest and lowest levels from the first batch prepared. Satisfactory stability was shown at both dose levels over intervals of up to 10 weeks (99.1 to 102.2% of initial concentration).

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
The incidence of clinical findings was low and none was unequivocally related to treatment. There was a low incidence of diarrhoea (during the second week of the study) in the group receiving 20000 ppm glyphosate acid. The faeces of both sexes at this dose level were observed to be paler than those of control or other test groups.

C. BODY WEIGHT
No relevant differences in the mean body weight gain were noted between controls and animals given 1000 or 5000 ppm. Body weight gain was reduced in male rats fed 20000 ppm glyphosate acid from the first week of the

study. The body weights continued to diverge from control values as the study progressed, and final body weights were approximately 8% lower than those of controls (see Table 6.3.2.1-3). Final body weights and body weight gains of females in all dose groups were similar to controls.

Table 6.3.2-2: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted] 1996): Intergroup comparison of body weight gain – selected time points from start of study

	Mean cumulative body weight gain [g]							
	Initial weight	**Week 1**	**Week 2**	**Week 4**	**Week 7**	**Week 10**	**Week 13**	**Final weight**
Dose [ppm]	**Males**							
0	135.0	51.8	104.0	185.5	254.6	305.1	333.3	468.3
1000	↑140.3	↑54.3	↑106.3	↑187.5	↓253.4	↓304.9	↓327.0	↓467.3
5000	↑136.3	51.8	↓103.4	↑186.1	↑255.1	↑306.3	↓331.9	468.3
20000	↓134.5	**↓45.1** ** **(-13%)**	**↓94.0*** **(-10%)**	**↓166.9** ** **(-10%)**	**↓226.0** ** **(-11%)**	**↓272.00** * **(-11%)**	**↓295.8** ** **(-11%)**	**↓430.3** ** **(-8%)**
	Females							
0	121.3	26.6	47.3	81.6	112.8	130.8	143.3	264.6
1000	↑122.2	↑27.7	↑51.4	↑82.7	↑113.1	↑132.1	↑146.0	↑268.2
5000	121.3	↓25.9	↑50.2	↑82.9	↓110.0	↓129.5	↓138.4	↓259.8
20000	↓118.6	↓24.3	**↑53.5***	↑83.3	↑115.1	↑132.7	↓142.5	↓261.1

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided);
** Statistically significant from controls, $p < 0.01$ (Student´s t-test, 2-sided)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

The food consumption of males fed 20000 ppm glyphosate acid was reduced from the fifth week of the study compared to control values but the reduction was small and did not attain statistical significance in any week. The food utilisation efficiency of males at this dose level was reduced throughout the study. The food consumption and food utilisation efficiency of males fed 1000 or 5000 ppm glyphosate acid and of females at all dose levels were similar to those of controls.

Table 6.3.2-3: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats ([redacted] 1996): Intergroup comparison of food consumption [g/rat/day] – selected time points from start of study

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Weeks	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
1	22.9	↑23.9	↑23.7	↓22.6	17.8	↑17.9	↓17.5	17.8
5	27.2	↓26.6	↑27.3	↓25.5	19.0	19.0	↓18.2	↓18.5
9	28.5	↓28.3	28.5	↓26.9	19.9	↑20.0	↓19.5	19.9
13	24.9	↓23.7	↓24.3	↓22.8	17.6	↑18.0	↓17.4	17.6

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided)

Table 6.3.2-4: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Intergroup comparison of food utilisation [g growth/100 g food] – selected time points from start of study

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Weeks	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
1-4	25.15	↓24.85	↓24.99	**↓22.89***	15.73	↓15.42	↑15.78	↑15.91
5-8	11.25	↓11.09	↑11.36	↓10.31	7.49	↓6.74	↓6.71	↓7.29
9-13	6.45	↓5.76	↓6.05	↓5.88	3.08	↑3.95	↑3.10	↓2.96

Table 6.3.2-4: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Intergroup comparison of food utilisation [g growth/100 g food] – selected time points from start of study

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Weeks	0	1000	5000	20000	0	1000	5000	20000
Overall (1-13)	13.59	↓13.30	↓13.44	↓**12.54***	8.28	↑8.34	↓8.14	↓8.25

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided)

Calculated mean test compound intakes are presented in the following table.

Table 6.3.2-5: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Overall mean test compound intake

	Males				Females			
Dietary concentration of glyphosate acid [ppm]	**0**	**1000**	**5000**	**20000**	**0**	**1000**	**5000**	**20000**
Achieved intake [mg/kg bw/day]	0	81.33	413.5	1612	0	90.42	446.9	1821

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period. The small incidence of findings recorded was within the normal background incidence for rats of this age and strain.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related effects noted in any dose group.

Blood clinical chemistry

The plasma activities of alanine transaminase (ALT) and alkaline phosphatase (ALP) were increased in both sexes fed 20000 ppm glyphosate acid throughout the study. Plasma aspartate transaminase activity was increased in females fed 20000 ppm glyphosate acid at week 4 only.

Plasma ALT activity was also increased in males receiving 5000 ppm glyphosate acid at weeks 4 and 13 and in females at week 4 only.

The plasma ALP activities of males receiving 5000 or 1000 ppm glyphosate acid were marginally increased. These increases were not dose-related and for the 1000 ppm glyphosate acid group were attributed to the high values in 3 out of 12 males. These marginal differences from the control group are considered to be of doubtful significance and not to be treatment-related.

Plasma urea levels were marginally decreased in both sexes at week 13 and in males at week 4 in the 20000 ppm glyphosate acid group.

Males receiving glyphosate acid showed marginal reductions in plasma glucose levels at week 4 but not at week 13. Females at 20000 ppm glyphosate acid showed a slight increase in this parameter at week 13 only.

Plasma cholesterol levels were unaffected by treatment with glyphosate acid. Plasma triglyceride levels were slightly reduced in males receiving 20000 ppm glyphosate acid at both weeks 4 and 13, the effect being greater at week 13.

Both males and females receiving glyphosate acid showed marginal reductions in plasma albumin and total protein. The changes were not consistent, showed no dose-response relationship and are therefore considered to be of dubious significance.

Table 6.3.2-6: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Intergroup comparison of selected clinical chemistry parameters

Parameter	Week	Dietary concentration of glyphosate acid [ppm]							
		Males				Females			
		0	1000	5000	20000	0	1000	5000	20000
ALT [mU/mL]	4	61.0	66.8	**76.0****	**83.8****	47.3	50.8	**57.7***	**73.8****
	13	51.9	52.3	**62.3***	**65.2****	45.0	45.2	46.2	**55.0****
ALP [mU/mL]	4	273	**326****	**320***	**411****	188	199	212	**309****
	13	148	159	**176***	**215**** (+45%)	91	94	99	**140**** (+54%)
ASAT [mU/mL]	4	62.8	67.0	69.1	68.5	56.0	57.0	57.5	**64.8****
	13	52.7	52.3	56.4	52.4	53.1	49.8	52.8	52.6
Urea [mg/100mL]	4	47.0	45.8	44.6	**43.6***	45.8	45.3	46.8	44.2
	13	41.9	39.9	40.0	**37.7***	40.6	40.1	42.1	**35.9***
Glucose [mg/100mL]	4	143	**133***	**132***	**128****	141	146	142	136
	13	191	186	208	197	182	183	183	**208****
Triglycerides [mg/100mL]	4	151	145	147	136	80	75	88	84
	13	153	157	144	**120****	72	74	77	77
Albumin [g/100 mL]	4	4.68	4.67	4.64	4.67	4.63	4.57	**4.49***	4.58
	13	4.81	**4.60***	4.82	**4.62***	4.54	4.42	4.42	4.42
Total Protein [g/100 mL]	4	6.13	6.15	6.12	6.13	5.84	5.88	5.80	5.87
	13	6.53	**6.22****	6.43	**6.06****	5.84	5.79	5.82	5.69

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided);
** Statistically significant from controls, $p < 0.01$ (Student´s t-test, 2-sided)

G. URINALYSIS

There were no treatment-related findings.

H. NECROPSY

Organ weights

Absolute heart weight of top dose males was reduced compared to controls but the reduction reflected the reduced body weight. There were no other differences in organ weights which were considered to be related to treatment.

Table 6.3.2-7: First Revision to Glyphosate Acid: 90 Day Feeding Study in Rats [redacted] 1996): Intergroup comparison of heart weights [grams]

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	1000	5000	20000	0	1000	5000	20000
Absolute	1.310	↑1.331	↓1.296	↓**1.197***	0.881	↑0.889	↑0.892	↑0.910
Adjusted for body weight	1.283	↑1.308	↓1.269	↓1.274	0.878	↓0.877	↑0.901	↑0.915

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided)

Gross pathology

A small number of lesions were observed, none of which was related to treatment.

Histopathology

There were no histopathological findings related to treatment. The incidence of findings was low and, with one exception, of a type commonly found in rats of this strain and age. An uterine leiomyosarcoma was seen in a female fed 5000 ppm glyphosate acid. Whilst the occurrence of a malignant tumour of smooth muscle in the uterus of a young rat is unusual, this isolated finding in an intermediate dose group is considered not to be related to treatment.

III. CONCLUSIONS

Males fed 20000 ppm glyphosate acid showed small reductions in body weight gain, food consumption and food utilisation efficiency compared to controls. There were associated minor reductions in plasma urea, total protein and triglycerides. Increased levels of plasma ALT and ALP were seen in both male and female rats at the top dose and are considered to indicate an altered liver metabolism but there was no evidence of histological change in this organ.

Effects in rats fed 5000 ppm glyphosate acid were confined to marginal increases in plasma ALT levels in females and in both plasma ALT and ALP levels in males. The magnitude and isolated nature of these effects leads them to be considered of no biological significance.

Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry. The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change.

Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats. The minor changes in clinical chemistry seen at 5000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the no-effect level for glyphosate acid in this study.

Assessment and conclusion by applicant:
In this study, groups of twelve male and twelve female Alpk:AP (now known as Alpk:APfSD) Wistar-derived rats were fed diets containing 0 (control), 1000, 5000 or 20000 ppm glyphosate acid for 90 consecutive days in accordance with OECD 408 (1998) and in compliance with GLP (no certificate of the competent authority was provided).
Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry. The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change.
Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats. The minor changes in clinical chemistry seen at 5000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the NOAEL for glyphosate acid in this study (equivalent to 413.5 mg/kg bw/day for males and 446.9 mg/kg bw/day for females).

Assessment and conclusion by RMS:
The RMS considers this study as reliable as the deviations from the current version are due to the fact that the study was aligned to an older version of OECD TG 408. The RMS agrees with the assessment of the applicant and the derived NOAEL of 5000 ppm glyphosate acid (equivalent to 413.5 mg/kg bw/day for males and 446.9 mg/kg bw/day for females). At the LOAEL of 20000 ppm, body weight gain was reduced in males and alkaline phosphatase was increased in both males and females (>50% compared with controls).

This conclusion is in line with previous assessment in the RAR (2015) in which the following was concluded: " This study is considered acceptable and the conclusions, including setting of the NOAEL, are agreed with. The significant changes in clinical chemistry parameters at the mid dose level in males might be indicative of a weak effect on the liver, however, since they were not accompanied by histological lesions and/or liver weight increase, are not regarded as adverse. In addition to the study description above, it should be noted that, as in previous studies with glyphosate, urine pH was significantly decreased in top dose males and in mid and high dose females. From the study report it seems that salivary glands were taken but neither weighed nor examined microscopically although they were a target organ in other studies"

B.6.3.2.2. Oral 90-day toxicity study in rats – study 2

Data point	CA 5.3.2/003
Report author	[redacted]
Report year	1996
Report title	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat

Report No	434/016
Document No	Not reported
Guidelines followed in study	JMMAF 59 NohSan No. 4200; data from the study report is equivalent to OECD 408.
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted; cholesterol not measured, no blood hormones (T3, T4 and TSH) measured; thymus, uterus, epididymis, prostate and seminal vesicles not weighed with testes; epididymis, coagulating glands not examined microscopically and spinal cord only examined at one level; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. In addition, the limit dose of 1000 mg/kg bw/day was exceeded. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary

The test material was administered by dietary admixture to three groups, each of ten male and ten female Sprague Dawley (CD) strain rats, for ninety consecutive days, at dietary concentrations of 1000, 10000 or 50000 ppm (equivalent to an estimated mean achieved dose level of 79, 730 or 3706 mg/kg bw/day for males and 90, 844 or 4188 mg/kg bw/day for females). A further group of ten males and ten females was exposed to basal laboratory diet to serve as a control.

Clinical signs, body weight, food and water consumption were monitored during the study. Haematology, blood chemistry and urinalysis were evaluated for all animals at the end of the study. Ophthalmoscopic examination was also performed. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed.

At 1000 ppm no treatment-related effects were noted in any of the investigation conducted.

In the mid dose group statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase was observed in both sexes. Histopathology revealed a mucosal atrophy of the caecum in this group. No other treatment-related findings were observed in this dose group.

Animals treated with 50000 ppm showed soft faeces/diarrhoea from Day 4 which continued throughout the study period. In addition, body weight gain, food intake and food efficiency in animals of both sexes in the high-dose group was reduced over the first four weeks of treatment when compared with controls. Body weight development, food consumption and efficiency recovered in females and were comparable with the control group by the end of the treatment period. In males body weight gain showed only a partial recovery, and an adverse effect on dietary intake was still apparent during the remaining treatment period. Animals of both sexes treated with 50000 ppm showed a statistically significant reduction in plasma calcium concentration and creatinine levels, as well as an increase in alkaline phosphatase and inorganic phosphorous in comparison with controls. Reductions in total protein and albumin were observed only in high-dose females. Urinalysis revealed increased levels of haemoglobin when compared with controls. Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated with 50000 ppm. At necropsy high dose animals of both sexes showed an enlarged and fluid-filled caecum, as well as statistically increased liver and kidney weights. Microscopic examination of the caecum revealed changes identified as mucosal atrophy for animals of both sexes treated with 50000 or 10000 ppm. No treatment-related histopathological changes were detected in the 1000 ppm dose group.

The NOAEL is considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females) based on atrophy in the caecum in one male and two females at the mid dose of 10000 ppm.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Technical Glyphosate
Description:	White powder
Lot/Batch#:	H95D161A
Purity:	95.3%
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	6 – 7 weeks
Sex:	Male and female
Weight at dosing:	♂ 175 – 218 g; ♀ 145 – 195 g
Acclimation period:	7 days
Diet/Food:	Rat and Mouse SQC Ground Diet No.1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of up to four by sex in polypropylene grid-floor cages.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 55 ± 15% Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-08-11 to 1996-01-30

Animal assignment and treatment:
In a 90 day feeding study groups of 10 Sprague-Dawley rats per sex received daily dietary doses of 0, 1000, 10000 or 50000 ppm (equivalent to mean achieved dose levels of 0, 79, 730 or 3706 mg/kg bw/day for males and 0, 90, 844, or 4188 mg/kg bw/day for females) technical glyphosate in the diet.
Test diets were prepared prior to start of treatment and then twice during the three month study period 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 homogeneity and achieved concentration.

Clinical observations
A check for clinical signs of toxicity, ill health and behavioural changes was made once daily on all animals. All observations were recorded.

Body weight
Individual body weights were recorded on Day 0 (prior to treatment) and at weekly intervals thereafter. Body weights were also determined at necropsy.

Food consumption and compound intake

Food consumption was recorded once weekly for each cage group throughout the study.

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

Ophthalmoscopic examination
The eyes of all control and high dose animals were examined before administration of the test and control diets and before termination of treatment (during Week 12). Examinations included observation of the anterior structures of the eye, pupillary and corneal blink reflex and, following pupil dilation with 0.5 % Tropicamide solution ("Mydriacyl" - Alcon Laboratories Ltd., Watford, Hertfordshire, UK), detailed examination of the internal structure of each eye using a direct ophthalmoscope.

Haematology and clinical chemistry
Haematological and blood chemical investigation were performed on all animals from each test and control group at the end of the study (Day 90).
Haematology parameters: Haemoglobin (Hb), Erythrocyte count (RBC), Haematocrit (Hct), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), Total leucocyte count (WBC), Differential leucocyte count (neutrophils (Neut), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eos), basophils (Bas)) and Platelet count (PLT)
Blood chemistry parameters: Urea, Glucose, Total protein, Albumin, Albumin/Globulin ratio (by calculation), Sodium (Na+), Potassium (K+), Chloride (Cl-), Calcium (Ca++), Inorganic phosphorus (P), Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), Alkaline phosphatase (AP), Creatinine (Creat) and Total bilirubin (Bili).

Urinalysis
Analytical investigations of the urine were performed on all animals during Week 12 and comprised the following parameters: volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilirubin, reducing substances, blood and microscopic examination of sediment. Urine samples were collected overnight by housing the rats in metabolism cages. Animals were maintained under conditions of normal hydration during collection but without access to food.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: adrenals, brain, gonads, heart, kidneys, liver, pituitary and spleen.

Tissue samples were taken from the following organs and preserved in buffered formalin: Adrenals, aorta (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (at three levels), caecum, colon, duodenum, eyes, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs, mammary gland, lymph nodes (cervical and mesenteric), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (hind limb), spinal cord (cervical), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, uterus and vagina.

Statistics
Absolute and relative organ weights, haematological and blood chemical data were analysed by one way analysis of variance incorporating 'F-max' test for homogeneity of variance. Data showing heterogeneous variances were analysed using Kruskal-Wallis non-parametric analysis of variance and Mann Whitney U-Test. The levels of probability chosen as significant were $p < 0.001$***, $p < 0.01$** and $p < 0.05$*.

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

II. RESULTS AND DISCUSSION

DIETARY ANALYSIS

The mean concentrations determined in all of the diets prepared for this study were within 90 to 113% of the nominal concentration. Satisfactory homogeneity of mixing was also demonstrated with values falling within ±5% of the overall mean concentration. Satisfactory stability was shown at all dose levels after storage for seven weeks (98-106% of initial concentration).

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

All animals of both sexes treated with 50000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.

The remaining observable sign of generalised fur loss was noted in one male and two females treated with 10000 and 1000 ppm respectively. This is a commonly reported incidental finding in laboratory maintained rats that, in the absence of any dose-related response, is of no toxicological significance and unrelated to treatment with the test material.

C. BODY WEIGHT

Animals of both sexes treated with 50000 ppm showed a reduction in body weight gain over the first four weeks of treatment when compared with controls (see table below). Female body weight development recovered as the study progressed and was comparable with the control group by the end of the treatment period. Male individuals showed only a partial recovery with body weight gain remaining slightly lower than the control group values during the subsequent weeks of treatment. Body weight development was unaffected by treatment with the test material at the remaining dose levels.

Table 6.3.2-1: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat [redacted] 1996): Group mean weekly body weights and standard deviations (SD)

Dietary concentration [ppm]		Body weight [g] at Day						
		0	7	14	21	28	35	42
Males								
0	mean	206	269	315	354	382	411	444
	SD	8	12	17	24	33	38	45
1000	mean	↓199	↓260	↓309	↓350	↓377	↓400	↓427
	SD	11	14	19	21	24	26	30
10000	mean	↓200	↓257	↓303	↓338	↓364	↓393	↓414
	SD	12	12	15	21	25	30	35
50000	mean	↓198	↓215	↓247	↓268	↓283	↓306	↓329
	SD	8	8	15	21	26	31	33
Females								
0	mean	173	197	214	232	243	256	269
	SD	9	11	12	15	16	18	20
1000	mean	173	↑199	↑218	↑238	↑249	↑261	↑272
	SD	10	13	14	16	16	17	18
10000	mean	↓166	↓184	↓201	↓217	↓226	↓237	↓246
	SD	14	18	21	25	24	26	27
50000	mean	173	↓183	↓197	↓214	↓219	↓231	↓240
	SD	11	12	14	15	14	18	21

Dietary concentration [ppm]		Body weight [g] at Day						
		49	**56**	**63**	**70**	**77**	**84**	**90**
Males								
0	mean	457	488	508	523	537	536	551
	SD	44	49	52	55	58	56	58
1000	mean	↓446	↓470	↓485	↓497	↓513	↓516	↓528
	SD	31	32	32	35	37	36	37
10000	mean	↓429	↓454	↓470	↓483	↓494	↓495	↓506
	SD	35	38	38	38	40	39	43
50000	mean	↓335	↓356	↓369	↓382	↓394	↓395	↓408 (-26%)
	SD	38	41	43	43	44	42	44
Females								
0	mean	276	284	291	295	306	304	307
	SD	19	20	21	24	25	25	27
1000	mean	↑280	↑286	↑292	↑300	↑308	304	↑313
	SD	19	18	18	19	21	20	20
10000	mean	↓256	↓262	↓267	↓272	↓277	↓276	↓282
	SD	27	27	27	27	29	28	29
50000	mean	↓246	↓251	↓260	↓265	↓271	↓267	↓273 (-11%)
	SD	21	20	23	23	26	22	25

D. FOOD CONSUMPTION

Animals of both sexes treated with 50000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls (see Table below). Female food consumption and efficiency recovered as the study progressed and was comparable with control values by the end of the treatment period. Male food consumption however, remained adversely affected during the subsequent weeks of treatment. A similar prolonged effect on food efficiency was not evident during the same period as male body weight gain demonstrated a partial recovery over the corresponding weeks.

Dietary intake and food efficiency were unaffected by treatment with the test material at the remaining dose levels and were comparable with controls.

Table 6.3.2-2: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted], 1996): Group mean weekly food consumption

Dietary concentration [ppm]	Mean food consumption [g/rat/week]						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Males							
0	201	199	204	212	208	218	208
1000	↓200	↑205	↑213	↓211	↓205	↓210	↑211
	(0)	(3)	(4)	(0)	(-1)	(-4)	(1)
10000	↓187	↓193	↓199	↓204	↓202	↓198	↓201
	(-7)	(-3)	(-2)	(-4)	(-3)	(-9)	(-3)
50000	↓122	↓183	↓178	↓177	↓183	↓182	↓168
	(-39)	(-8)	(-13)	(-17)	(-12)	(-17)	(-19)
Females							
0	140	131	171	153	149	149	152
1000	↑143	↑146	↓152	↑156	↑158	↑163	↑157
	(2)	(11)	(-11)	(2)	(6)	(9)	(3)
10000	↓123	↑135	↓142	↓144	↓143	↓140	↓143
	(-12)	(3)	(-17)	(-6)	(-4)	(-6)	(-6)
50000	↓128	↑143	↓131	↓148	↑167	↑157	↓148

Table 6.3.2-2: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted], 1996): Group mean weekly food consumption

Dietary concentration [ppm]	Mean food consumption [g/rat/week]						
	1	2	3	4	5	6	7
	(-9)	(9)	(-23)	(-3)	(12)	(5)	(-3)

Dietary concentration [ppm]	Mean food consumption [g/rat/week]					
	8	9	10	11	12	13*
Males						
0	222	224	223	214	192	185
1000	↓217	↓204	↓219	214	↓191	↓180
	(-2)	(-9)	(-2)	(0)	(-1)	(-3)
10000	↓205	↓211	↓211	↓201	↓185	↓179
	(-8)	(-6)	(-5)	(-6)	(-4)	(-3)
50000	↓187	↓189	↓193	↓188	↓174	↓171
	(-16)	(-16)	(-13)	(-12)	(-9)	(-8)
Females						
0	152	151	147	155	139	128
1000	↑159	↑152	↑154	↑161	↑141	↑137
	(5)	(1)	(5)	(4)	(1)	(7)
10000	↓146	↓143	↓143	↓142	↓133	↑131
	(-4)	(-5)	(-3)	(-8)	(-4)	(2)
50000	↓151	151	↑151	↑161	139	↑139
	(-1)	(0)	(3)	(4)	(0)	(9)
( ) % change compared to control group;						
* Week 13 comprises six days only						

E. WATER CONSUMPTION

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

F. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular effects for either sex were detected during the study.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

No treatment-related effects were detected in the haematological parameters measured.

Blood chemistry

Animals of both sexes treated with 50000 or 10000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls (see table below). A statistically significant increase in inorganic phosphorus and reduction in plasma creatinine were also evident amongst animals of both sexes treated with 50000 ppm whilst females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls.

There were no further treatment-related effects.

Table 6.3.2-3: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Group mean blood chemical values and standard deviations (SD)

Dietary concentration [ppm]		Ca^{2+} [mmol/L]	AP [IU/L]	P [mmol/L]	Creatinine [mg/dL]	Total protein [g/dL]	Albumin [g/dL]
Males							
0	mean	2.74	373	2.23	0.61	7.02	3.37
	SD	0.06	101	0.22	0.03	0.47	0.16

Table 6.3.2-3: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat [redacted] 1996): Group mean blood chemical values and standard deviations (SD)

Dietary concentration [ppm]		Ca^{2+} [mmol/L]	AP [IU/L]	P [mmol/L]	Creatinine [mg/dL]	Total protein [g/dL]	Albumin [g/dL]
Males							
1000	mean	↑2.77	↑404	↓2.22	↑0.62	↑7.20	↑3.40
	SD	0.07	115	0.16	0.05	0.20	0.06
10000	mean	**↓2.66***	**↑514* (+38%)**	↑2.32	↓0.59	↓6.78	↓3.30
	SD	0.09	106	0.28	0.04	0.68	0.21
50000	mean	**↓2.64***	**↑597*** (+60%)**	**↑2.46***	**↓0.57***	↓6.63	↓3.27
	SD	0.10	150	0.22	0.04	0.63	0.19
Females							
0	mean	2.78	230	1.70	0.69	7.63	3.90
	SD	0.11	38	0.33	0.07	0.45	0.23
1000	mean	↓2.76	↑261	↓1.65	0.69	↑7.64	↓3.87
	SD	0.05	71	0.21	0.04	0.29	0.13
10000	mean	**↓2.70***	**↑408*** (+77%)**	↑1.76	↓0.65	↓7.41	↓3.82
	SD	0.07	123	0.23	0.04	0.45	0.20
50000	mean	**↓2.56*****	**↑358** (+56%)**	**↑2.12*****	**↓0.61****	**↓6.86****	**↓3.47*****
	SD	0.10	90	0.15	0.05	0.82	0.39

* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$);
*** Significantly different from control group ($p < 0.001$)

H. URINALYSIS

Animals of both sexes treated with 50000 ppm showed an increased level of haemoglobin in the urine when compared with controls (see table below). Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated at 50000 ppm. This probably represents external contamination, possibly of faecal origin.

There were no treatment-related changes detected at the remaining dose levels.

Table 6.3.2-4: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat [redacted], 1996): Urinalysis findings

Dietary concentration [ppm]	Blood (haemoglobin)							
	Males				Females			
	-	+	++	+++	-	+	++	+++
0	8	0	1	1	10	0	0	—
1000	10	0	0	0	10	0	0	—
10000	7	2	1	0	10	0	0	—
50000	1	5	2	2	4	3	3	—

– negative;
+ ca. 5-10 × 10^6 ery/L;
++ ca. 50 × 10^6 ery/L;
+++ ca. 250 × 10^6 ery/L

I. NECROPSY

Organ weights

Animals of both sexes treated with 50000 ppm showed statistically significant increases in both relative liver and kidney weight when compared with controls (see table below).

There were no further direct effects of treatment.

Table 6.3.2-5: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat [redacted] 1996): Group mean organ weights and standard variations

Dietary concentration [ppm]		Relative organ weight [%]			
		Liver		Kidney	
		♂	♀	♂	♀
0	mean	2.9749	2.9734	0.5861	0.6516
	SD	0.2629	0.1558	0.0575	0.0523
1000	mean	↓2.8868	↓2.9093	↑0.5901	↓0.6257
	SD	0.2652	0.2146	0.0804	0.0375
10000	mean	↓2.8853	↑2.9801	↑0.6070	↓0.6454
	SD	0.3758	0.1556	0.0552	0.0532
50000	mean	**↑3.2433***	**↑3.1989***	**↑0.6963*** (+19%)**	**↑0.7180* (+10%)**
	SD	0.2452	0.2098	0.0436	0.0707

* Significantly different from control group ($p < 0.05$);
*** Significantly different from control group ($p < 0.001$)

Necropsy
Macroscopic abnormalities were detected in the 50000 ppm dose group with all animals showing an enlarged and fluid-filled caecum whilst one female treated with 50000 ppm showed gaseous distension of the stomach at terminal necropsy.

There were no treatment-related macroscopic abnormalities detected at 10000 or 1000 ppm.

Table 6.3.2-6: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat [redacted] 1996): Summary of necropsy findings

Finding	Dietary concentration [ppm]							
	Males				Females			
	0	1000	10000	50000	0	1000	10000	50000
Caecum: enlarged with fluid contents	0/10	0/10	0/10	10/10	0/10	1/10	0/10	10/10

Histopathology
Treatment-related changes were observed in the caecum. Atrophy, characterised by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50000 ppm ($p < 0.05$ for male rats) and for one male and two female rats receiving 10000 ppm of the test material. The aetiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from caecal distension.
There were no further treatment-related changes.

Table 6.3.2-7: Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study in the Rat ([redacted] 1996): Summary incidence of histopathological findings

Finding	Dietary concentration [ppm]							
	Males				Females			
	0	1000	10000	50000	0	1000	10000	50000
Caecum: Mucosal atrophy	0/10	0/10	1/10	5/10	2/10	0/10	2/10	5/10

III. CONCLUSIONS

At 1000 ppm no treatment-related effects were noted in any of the investigation conducted.
In the mid dose group statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase was observed in both sexes. Histopathology revealed a mucosal atrophy of the caecum in this group. No other treatment-related findings were observed in this dose group.

Animals treated with 50000 ppm showed soft faeces/diarrhoea from Day 4 which continued throughout the study period. In addition, body weight gain, food intake and food efficiency in animals of both sexes in the high-dose group was reduced over the first four weeks of treatment when compared with controls. Body weight development, food consumption and efficiency recovered in females and were comparable with the control group by the end of the treatment period. In males body weight gain showed only a partial recovery, and an adverse effect on dietary intake was still apparent during the remaining treatment period. Animals of both sexes treated with 50000 ppm showed a statistically significant reduction in plasma calcium concentration and creatinine levels, as well as an increase in alkaline phosphatase and inorganic phosphorous in comparison with controls. Reductions in total protein and albumin were observed only in high-dose females. Urinalysis revealed increased levels of haemoglobin when compared with controls. Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated with 50000 ppm. At necropsy high dose animals of both sexes showed an enlarged and fluid-filled caecum, as well as statistically increased liver and kidney weights. Microscopic examination of the caecum revealed changes identified as mucosal atrophy for animals of both sexes treated with 50000 or 10000 ppm. No treatment-related histopathological changes were detected in the 1000 ppm dose group.

Dietary administration of the test material, technical glyphosate, to rats for a period of 90 consecutive days at concentrations of up to 50000 ppm, resulted in treatment-related changes at 50000 and 10000 ppm. No such effects were demonstrated in the 1000 ppm treatment group and the "No Observed Effect Level" was, therefore, considered to be 1000 ppm.

Assessment and conclusion by applicant:
In this study, glyphosate technical was administered via the diet to three groups, each of ten male and ten female Sprague Dawley (CD) strain rats, for 90 consecutive days, at dietary concentrations of 1000, 10000 or 50000 ppm (equivalent to mean achieved dose level of 79, 730 or 3706 mg/kg bw/day for males and 90, 844 or 4188 mg/kg bw/day for females).

Dietary administration of the test material, technical glyphosate, to rats for a period of 90 consecutive days at concentrations of up to 50000 ppm, resulted in treatment-related changes at 50000 and 10000 ppm. No such effects were demonstrated in the 1000 ppm treatment group and the NOAEL was, therefore, considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

Assessment and conclusion by RMS:
The RMS considers this study as acceptable as the deviations from the current version are due to the fact that the study was aligned to an older version of OECD TG 408. The conclusions drawn are supported by the RMS, which was also concluded by the RMS DE in the previous assessment of glyphosate (see below).

In this study, at the top dose of 50000 ppm soft faeces and diarrhoea was noted in all animals of both sexes. In addition, at this dose level an adverse and treatment-related decreased body weight was noted in males and females. Body weight gain was not reported. Food consumption was reduced in males, but not in females. The increased relative kidney weight was considered treatment-related and adverse in both sexes. Liver weight increases were not considered as adverse as weight changes were less than 15% compared with controls and as there were no histopathological findings in the liver. At the top dose, an increased alkaline phosphatase level was seen in both sexes, which was considered adverse (males +60%; females +56%). Slight effects were seen in other blood chemistry parameters. In addition, treatment-related changes were observed in the caecum which was and enlarged and filled with fluid in all animals of both sexes and atrophy of the caecum characterised by flattening of the intestinal mucosa in five out of ten rats of both sexes. As this atrophy in the caecum was also seen in one male and two females at the mid dose of 10000 ppm, this dose level is considered the LOAEL. At the mid dose, also an increase was seen in alkaline phosphatase levels in females (+77%), which was also considered adverse.
The NOAEL is considered to be 1000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

As already noted in the previous assessment in the RAR (2015), in this study a relative low NOAEL compared to other studies has been derived which is mainly due to the large dose spacing in the study (factor 10 between low and mid dose).

In the previous assessment in the RAR (2015), the following was concluded by the RMS DE:
"*The study is considered acceptable and the conclusions drawn are supported. The data submitter/owner was not mentioned in the study description but is presumed to be Nufarm. According to the study report itself, the sponsor was Mastra Industries (Malaysia) that gave Nufarm access to this study.*
The NOAEL of 79 mg/kg bw/day is agreed with. It must be kept in mind that, due to dose spacing, the margin between the low and mid dose level was very large. The LOAEL of 730 mg/kg bw/day was well above the NOAELs in other 90-day studies. Thus, despite the (relatively) low NOAEL, this study is not suitable to prove that toxicity of glyphosate was in fact higher than previously assumed. It must be also emphasised that the top dose level of 3700 mg/kg bw/day was indeed extremely high, even for this rather non-toxic compound.
Unfortunately, it is not clear from the report whether or not salivary glands (and if, which of them) were subject to histopathology.
Organ weight changes (including higher relative testis weight in addition to liver and kidney) at the top dose level are considered secondary to reduced body weight"

B.6.3.2.3. Oral 90-day toxicity study in rats – study 3

Data point	CA 5.3.2/004
Report author	[redacted]
Report year	1995
Report title	HR-001: 13-week Subchronic Oral Toxicity Study in Rats
Report No	[redacted] 94-0138
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No. 4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted, clotting not evaluated, total cholesterol but not HDL and LDL measured, urea not measured, no blood hormones (T3, T4 and TSH) measured; organ weights limited to brain, liver, kidneys, testes, adrenals and cecum; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408. Further, the use of three different batches of the test compound with a different purity was also considered a deviation by AGG.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary
A sub-chronic oral toxicity study of HR-001 was conducted in Sprague-Dawley (Crj:CD) rats of both sexes. The test substance was administered to the rats (12 animals/group/sex) by incorporating it into the basal diet at dose levels of 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195 2, 637 or 1892 mg/kg bw/day for females) for a period of 13 weeks (91 days).

30000 ppm group: Body weights of males and females were slightly lower than in the control throughout the treatment period and statistically significant decreases were sporadically observed. The averaged food efficiency

in males and females during the treatment period was slightly lower than that in the control. Females showed a significant increase in alkaline phosphatase (ALP) activity. Distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. Both sexes showed significant increases in absolute and relative weights of the caecum (containing contents). Histologically, there were no abnormalities related to treatment in any tissues including the caecum. In addition, relative liver weight was increased in females.

10000 ppm group: At necropsy, 3 males showed distention of the caecum. Organ weight analysis revealed a statistically significant increase (females) or an increasing trend (males) in both absolute and relative weights of the caecum.

3000 ppm group: There were no abnormalities attributable to the treatment in either sex.

The effects on the caecum reported at 10000 ppm are considered treatment-related and adverse. Based on these findings, the NOAEL is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	HR-001		
Description:	White crystal		
Lot/Batch#:	940908-1	941209	T-941209
Purity:	95.68 %	95.0 %	97.56 %
Stability of test compound:	12/12/1994	19/12/1994	26/12/1994

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crj:CD
Source:	[redacted]
Age:	5 weeks
Sex:	Male and female
Weight at dosing:	♂ 136 – 150 g; ♀ 109 – 121 g
Acclimation period:	1 week
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd.)
Water:	Filtered and sterilised tap water, *ad libitum*
Housing:	3/cage, sexes separately in stainless steel cages 31.0 × 44.0 × 20.3 cm
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: 1994-12-06 to 1995-03-22

Animal assignment and treatment:
The test substance was incorporated into the basal effect diet and administered on a continuous basis in the basal diet to groups of 24 Sprague-Dawley rats (12 males + 12 females) for a period of 13 weeks. Dietary concentrations were 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females).

Table 6.3.2-1: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Study design

Test group	Dietary concentration [ppm]	Achieved concentration [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	12	12
Low	3000	♂: 168.4; ♀: 195.2	12	12
Mid	10000	♂: 569; ♀: 637	12	12
High	30000	♂: 1735; ♀: 1892	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level taken from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weight was recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly over a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the food consumption by the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean chemical intake (mg/kg bw/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a halogen ophthalmoscope were performed on all animals during acclimatization period and on all surviving animals in the control and the highest dose groups from the main group at week 13.

The following parameters were determined: eyeball, cornea, anterior chamber, pupil, iris, lens/vitreous body and fundus.

Haematology and clinical chemistry

After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomised under anaesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.

The following parameters were determined with a fully automated haematology analyser: Haematocrit (Ht), haemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.

The following parameters were determined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).

Urinalysis
At 13 weeks of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semi-quantitatively analysed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy (brain, liver, kidneys, adrenals, caecum (containing contents), testes). Histopathological examinations were performed on selected tissues from all animals.

The following parameters were determined for histopathological changes: Brain (cerebrum, cerebellum, pons and medulla), spinal cord (cervical, thoracic and lumbar regions), sciatic nerve (unilateral), pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow (sternum; femur, unilateral; cervical, thoracic, and lumbar vertebrae), tibio-femoral joint (unilateral), lymph nodes (cervical and mesenteric), heart, aorta, pharynx, buccal mucosa of oral cavity, salivary glands (submaxillary and mesenteric), oesophagus, stomach (forestomach and glandular stomach), liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, head, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus (horns and cervix), vagina, harderian glands, eyes, skeletal muscle (M. triceps surae, unilateral), skin (lumbodorsal region, females only), mammary gland (abdominal region) and all gross lesions.

Statistics
All data were evaluated using variance analysis (body weight, food consumption, urine specific gravity, urine volume, haematological parameters, blood chemical parameters, and organ weights). 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 Scheffe's (when sample size of each group was different) multiple comparison test was applied to evaluate differences between the treated and 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 Scheffe 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 control groups.

Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

DIETARY ANALYSIS
The mean concentrations determined in all of the diets prepared for this study were within 91 to 108% of the target concentration. Satisfactory homogeneity of mixing was also demonstrated by a coefficient of variation for each dose level of 1.3% or less.

A. MORTALITY
No deaths were noted in the control and treated groups of either sex.

B. CLINICAL OBSERVATIONS
There were no abnormalities related to the treatment in clinical signs in the treated groups of either sex. In the 30000 ppm group, one female showed a poor general condition including emaciation and decreased spontaneous motor activity. The poor general condition seemed to be caused by elongated incisor, malocclusion, or hepatorenal genetic lesions revealed by histopathology. Thus, it was not considered to be treatment related.

C. BODY WEIGHT
In the 30000 ppm group, body weights of males and females were slightly lower (about 5 – 10% decrease in males and 5% in females) than those in the control throughout the treatment period. Statistically significant decreases in their body weights were sporadically observed during the treatment period (weeks 3, 4 and 11 in males and weeks 10 and 11 in females) when compared to the control. It should be noted that the calorific value of the diet would have been lower due to the incorporation, 3% by weight, of HR-001 (Glyphosate).

In the 10000 and 3000 ppm groups, body weight changes in males and females were comparable to the control throughout the treatment period.

Table 6.3.2-2: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Group mean weekly body weights (selected weeks) and standard deviations (SD)

Dietary concentration [ppm]		Body weight [g] at week					
		0	**3**	**4**	**10**	**11**	**13**
Males							
0	mean	142	314	351	503	519	541
	SD	4	23	30	47	48	50
3000	mean	142	↓310	↓348	↓502	↓516	↓540
	SD	4	20	24	45	49	53
10000	mean	142	↓311	↑352	↓501	↓517	541
	SD	4	21	29	41	45	48
30000	mean	142	**↓291* (-7%)**	**↓323* (-8%)**	↓462 (-8%)	**↓468* (-10%)**	↓497 (-8%)
	SD	4	19	30	50	53	53
Females							
0	mean	113	196	216	298	304	310
	SD	3	10	12	15	19	22
3000	mean	113	↑201	↑225	↑306	↑316	↑325
	SD	3	12	15	25	28	32
10000	mean	113	↑201	↑220	↓291	↓298	↓306
	SD	3	11	12	16	17	17
30000	mean	113	↓194	↓212	**↓278***	**↓282***	↓290
	SD	3	11	13	18	19	19

* Significantly different from control group ($p < 0.05$)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
In the 30000 ppm group, males and females showed significant decreases in food consumption at week 1 which were 9 and 14% lower than that of the control, respectively. However, their food consumption was comparable to the control at week 2 and thereafter.

In the 10000 ppm group no significant change was observed while in the 3000 ppm group, significant changes were sporadically observed during the treatment period in females during the weeks 6 and 7. The food consumption recovered from the week 8 up to the end of the study.
The overall food consumption by males and females was comparable to the control and there were no abnormalities considered to be treatment related.

In the 30000 ppm group, the averaged food efficiency for males and females during the treatment period was 6% and 5% less than that for control, respectively. This in part would have been accounted for by the 3% by weigh inclusion of HR-001 in the diet. Food efficiency for males and females was comparable to control in the 10000 and 3000 ppm groups.

Table 6.3.2-3: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Average food efficiency during the treatment period [body weight gain/food consumption × 100]

Dose level [ppm]	Average food efficiency [%]	
	Males	Females
0	20.7	14.6
3000	20.9	14.8
10000	20.7	14.0
30000	19.5	13.9

The overall group mean chemical intakes averaged, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, are summarised in the table below.

Table 6.3.2-4: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Chemical Intake [mg/kg bw/day]

Dose [ppm]	Chemical Intake [mg/kg bw/day]	
	Males	Females
3000	168.4	195.2
10000	569	637
30000	1735	1892

E. OPHTHALMOSCOPIC EXAMINATION
In the ophthalmological examination performed on all animals before the start of the treatment and on the animals of the control and 30000 ppm groups at 13 weeks of treatment, no abnormalities were observed in either sex.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology
There were no abnormalities in any group of either sex.

Blood clinical chemistry
In the 30000 ppm group, females showed a significant increase in alkaline phosphatase (ALP) activity and a significant decrease in albumin (Alb). There were no abnormalities in males.

In the 10000 and 3000 ppm groups, there were no abnormalities in either sex.

Table 6.3.2-5: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Intergroup comparison of selected week 13 clinical chemistry parameters

Parameter		Dietary concentration [ppm]							
		Males				Females			
		0	3000	10000	30000	0	3000	10000	30000
ALP [U/L]	mean	89	84	103	106	38	37	48	**69** (+82%)**
	SD	19	20	20	13	8	9	11	37

Table 6.3.2-5: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Intergroup comparison of selected week 13 clinical chemistry parameters

Parameter		Dietary concentration [ppm]							
		Males				Females			
		0	3000	10000	30000	0	3000	10000	30000
Alb [g/dL]	mean	3.15	3.12	3.08	3.14	3.78	3.82	3.70	**3.40***
	SD	0.12	0.09	0.18	0.15	0.23	0.36	0.29	0.35

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

In the 30000 ppm group, urine pH in males and females was significantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly higher urine volume than that of the control, but males showed a decreasing trend in urine volume as compared with the control.

In the 10000 ppm group, urine, pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter.

In the 3000 ppm group, no statistically significant changes were observed in either sex.

H. NECROPSY

Organ weights

In the 30000 ppm group, both sexes showed significant increases in absolute and relative weights of the caecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver.

In the 10000 ppm group, the absolute and relative weight of the caecum showed a statistically significant increase in males and increasing trend in females.

In the 3000 ppm group, there were no abnormalities attributable to the treatment in either sex.

Table 6.3.2-6: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Intergroup comparison of selected organ weights – absolute and relative to body weight

Organ		Dietary concentration [ppm]							
		Males				Females			
		0	3000	10000	30000	0	3000	10000	30000
Caecum	Absolute [g]	2.823 ± 0.794	↑3.187 ± 0.609	3.383 ± 1.081 (+20%)	**5.854** ± **2.053 (+106 %)**	2.367 ± 0.582	2.586 ± 0.462	**3.546* ± 0.959 (+50%)**	**5.268** ± **1.189 (+123 %)**
	Relative [%]	0.55 ± 0.16	0.62 ± 0.13	0.64 ± 0.20 (+16%)	**1.22** ± **0.41 (+122 %)**	0.79 ± 0.17	0.84 ± 0.17	**1.22* ± 0.32 (+54%)**	**1.92** ± **0.41 (+143 %)**
Brain	Absolute [g]	2.166 ± 0.088	2.122 ± 0.077	2.174 ± 0.078	2.125 ± 0.094	1.975 ± 0.106	1.999 ± 0.077	2.013 ± 0.059	1.965 ± 0.061
	Relative [%]	0.42 ± 0.04	0.41 ± 0.03	0.42 ± 0.03	0.45 ± 0.04	0.66 ± 0.06	0.65 ± 0.06	0.70 ± 0.05	**0.72* ± 0.04**
Liver	Absolute [g]	13.11 ± 1.48	12.69 ± 1.25	14.05 ± 2.01	12.95 ± 1.99	7.14 ± 0.44	7.38 ± 0.66	7.11 ± 0.59	7.77 ± 2.72 (+9%)

Table 6.3.2-6: HR-001: 13-week Subchronic Oral Toxicity Study in Rats [redacted] 1995): Intergroup comparison of selected organ weights – absolute and relative to body weight

Organ		Dietary concentration [ppm]							
		Males				Females			
		0	3000	10000	30000	0	3000	10000	30000
	Relative [%]	2.53 ± 0.16	2.45 ± 0.11	2.70 ± 0.30	2.73 ± 0.24	2.40 ± 0.19	2.37 ± 0.08	2.45 ± 0.18	**2.85* ± 1.06 (+19%)**

* Statistically significant from controls ($p < 0.05$);

** Statistically significant from controls ($p < 0.01$)

Gross pathology
In the 30000 ppm group, distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment.

In the 10000 ppm group, 3/12 males showed distention of the caecum, but there were no macroscopic abnormalities in females.

In the 3000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex.

Histopathology
Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male in the 10000 ppm group and one female in the 30000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

III. CONCLUSIONS

30000 ppm group: Body weights of males and females were slightly lower than in the control throughout the treatment period and statistically significant decreases were sporadically observed. The averaged food efficiency in males and females during the treatment period was slightly lower than that in the control. Females showed a significant increase in alkaline phosphatase (ALP) activity. Distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. Both sexes showed significant increases in absolute and relative weights of the caecum (containing contents). Histologically, there were no abnormalities related to treatment in any tissues including the caecum.

10000 ppm group: At necropsy, 3 males showed distention of the caecum. Organ weight analysis revealed a statistically significant increase (females) or an increasing trend (males) in both absolute and relative weights of the caecum.

3000 ppm group: There were no abnormalities attributable to the treatment in either sex.

Based on these results, the no-adverse-effect level, minimum toxic level, and sure toxic level of HR-001 in Sprague-Dawley (Crj:CD) rats under the conditions of the present study were determined as follows:

	Males	Females
No-adverse-effect level (NOAEL)	3000 ppm (168.4 mg/kg bw/day)	3000 ppm (195.2 mg/kg bw/day)
Minimum toxic level	10000 ppm (569 mg/kg bw/day)	10000 ppm (637 mg/kg bw/day)
Sure toxic level	30000 ppm (1735 mg/kg bw/day)	30000 ppm (1892 mg/kg bw/day)

Assessment and conclusion by applicant:

In this study, groups of male and female Sprague-Dawley (Crj:CD) rats were administered glyphosate technical via the diet at dose levels of 0, 3000, 10000 or 30000 ppm (equivalent to 0, 168.4, 569 or 1735 mg/kg bw/day for males and 0, 195.2, 637 or 1892 mg/kg bw/day for females) for a period of 13 weeks.

Under the experimental conditions of the study, the NOAEL is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:

The RMS considers this study as acceptable as the deviations from the current version are due to the fact that the study was aligned to an older version of OECD TG 408. The conclusions drawn are supported by the RMS, which was also concluded by the RMS DE in the previous assessment of glyphosate (see below).

In this study, males treated at 30000 ppm had a decreased body weight compared with controls throughout the study period. As body weight was decreased by >10% at several time points, this was considered as treatment-related and adverse. At the same dose level, also in females the body weight was decreased compared with controls, however, this was not considered adverse by the RMS as changes were less than 10% compared to controls. In both sexes, food consumption was decreased in the first week at the 30000 ppm dose level in both sexes, which was considered treatment-related and adverse. In females, blood alkaline phosphatase was increased at the 30000 ppm dose level. Distention of the caecum was seen in the majority of the males and females at the top dose, which was also reflected in an increased caecum weight at this dose level. Also at the mid dose, 3/12 males showed distension of the caecum and caecum weight was increased in both males and females. The effects on the caecum are considered treatment-related and adverse. In addition, relative kidney weight was significantly increased in females at 30000 ppm (+19% compared with controls), which is considered treatment-related and adverse. Based on these findings, the NOAEL is considered to be 3000 ppm (equivalent to 168.4 and 195.2 mg/kg bw/day for males and females, respectively).

In the previous assessment in the RAR (2015), the following was concluded by the RMS DE:
"*The study is considered acceptable and the proposed NOAEL is agreed with. Toxicity became apparent by the caecum findings and alterations in few clinical chemistry and urine parameters. The LOAEL of 569 mg/kg bw/day (10000 ppm) is higher than the NOAEL as established in other studies and, thus, does not contradict the previous assessment. From the study report, it became clear that submaxillary and sublingual salivary glands were histologically examined, without evidence of pathological changes. These glands were not weighed and parotid gland was not taken.*"

B.6.3.2.4. Oral 90-day toxicity study in rats – study 4

Data point	**CA 5.3.2/005**
Report author	[redacted]
Report year	1993 (Vol. 1, Study Report)
Report title	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 1)
Report No	011-0001
Document No	Not reported
Guidelines followed in study	No guideline stated, but in accordance with OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted; blood clotting not evaluated; total cholesterol but not HDL and LDL, T3, T4 and TSH evaluated; no blood hormones measured; prostate, uterus, thymus, pituitary, thyroids not weighed; seminal vesicles and coagulating glands were not examined microscopically; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older (1981) version of the OECD test guideline 408. Further, it is noted that blood chemistry was not carried out in some cases because the samples were inadvertently discarded.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point	**CA 5.3.2/006**
Report author	[redacted]
Report year	1993 (Vol. 2, Pathology Report)
Report title	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 2)
Report No	011-0001
Document No	Not reported
Previous evaluation	Yes, accepted in RAR (2015)
Data point	**CA 5.3.2/007**
Report author	[redacted]
Report year	1993 (Vol. 3, Histopathology Report)
Report title	90 Day Range Feeding Study of Glyphosate in Rats (Vol. 3)
Report No	011-0001
Document No	Not reported
Previous evaluation	Yes, accepted in RAR (2015)

Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary

In a subchronic toxicity study, groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0 (control), 2000, 6000 or 20000 ppm glyphosate acid (equivalent to 0, 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 0, 156.3, 481.2 or 1686.5 mg/kg bw/day for females) for 13 consecutive weeks.

Clinical observations were done daily. Body weights and food consumption was assessed weekly. Haematological, blood biochemistry parameters, as well as urine analysis were conducted prior to start of treatment and at termination. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem, and selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. The only treatment-related clinical finding was diarrhoea in all high-dose males and in 9 high-dose females. Mean body weights were comparable between all groups. The mean body weight gains were significantly different between groups at several time points, but the total mean body weight gain was similar in all dose groups. There were no differences in food consumption. There were no treatment-related findings in the haematological and clinical chemistry parameters. Urinalysis showed marginal increases in the mean scores for ketones, blood, protein and red blood cell counts in mid- and high-dose males, which were of no biological relevance. In mid- and high-dose females, microscopy revealed an increased number of rats with 1 red blood cell per high power field. Together with the appearance of blood in these groups this change seems to be treatment-related. The gross changes noted at necropsy were few and inconclusive with respect to association with exposure to the test article. The most prevalent abnormality was the non-treatment related swollen, reddened sublingual salivary glands. The mean weight of the adrenal glands in the high and low dose male groups was significantly decreased. The group mean weight of the spleen was significantly increased for the high dose female group when the spleen weights were compared as a percent of body weight. Gross and microscopic examinations did not identify tissue changes which were related to treatment. These findings are in agreement with clinical chemistry, hematologic and gravimetric data collected during the conduct of this study.

The results indicate that under the conditions of this investigation, dietary levels of up to 6000 ppm glyphosate (equivalent to 1262.1 mg/kg bw/day for males and 1686.5 mg/kg bw/day for females) were tolerated without significant pathophysiologic alterations. According to the RMS, this NOAEL is based on the occurrence of diarrhoea in both sexes, decreased body weight gain at day 50 and 85 in both sexes, decreased absolute and relative

adrenal weights in males, increased absolute and relative spleen weight in females and blood in urine at the LOAEL of 20000 ppm.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid (N-phosphonomethyl-glycine)
Description:	Greyish-white or yellowish-white crystalline powder
Lot/Batch#:	46540992
Purity:	97.5 %
Stability of test compound:	1994-10-01

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR VAF/Plus®)
Source:	[redacted]
Age:	approx. 5 weeks
Sex:	Male and female
Weight at dosing:	♂ 208.2 – 249.9 g; ♀ 159.8 – 202 g
Acclimation period:	14 days
Diet/Food:	Purina certified Laboratory Rodent Chow 5002, *ad libitum*, (except during collection of urine samples)
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages
Environmental conditions:	Temperature: 18 – 26 °C Humidity: 55 ± 15% Air changes: ≥10/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1993-02-17 to 1993-05-21

Animal assignment and treatment:
In a 90–day oral toxicity study groups of 10 Sprague-Dawley per sex received daily doses of 0, 2000, 6000 or 20000 ppm (equivalent to 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 156.3, 481.2 or 1686.5 mg/kg bw/day for females) in the diet. The test diets were prepared weekly and stored at room temperature. Samples of the control and test substance diets were analysed for stability and homogeneity. Dietary preparations were analysed for achieved concentrations weekly during the first 4 weeks, and then every fourth week thereafter.

Table 6.3.2-1: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Study design

Test group	Dietary concentration [ppm]	Achieved dose level [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	10	10
Low	2000	♂: 125.2; ♀: 156.3	10	10
Mid	6000	♂: 371.9; ♀: 481.2	10	10

Table 6.3.2-1: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Study design

Test group	Dietary concentration [ppm]	Achieved dose level [mg/kg bw/day]	Males	Females
High	20000	♂: 1262.1; ♀: 1686.5	10	10

The experimental diets were prepared weekly by adding the appropriate amount of glyphosate acid to the diet.

The homogeneity of glyphosate in the diet was determined by replicate samples from each dose level. Achieved concentrations were determined by analysing samples from each dose level during weeks 1 to 4, and every fourth week thereafter. The chemical stability of glyphosate acid in diet at room temperature was determined for all dose levels after preparation. Analysis was done by high performance liquid chromatography (HPLC).

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals.

Body weight
The body weight of each animal was recorded on Day 1 before start of treatment and weekly thereafter.

Food consumption and utilisation
Individual food consumption was recorded weekly.

Ophthalmoscopic examination
The eyes of all animals from all dose groups were examined prior to initiation and just prior to termination. The examination included macroscopic and ophthalmoscopic examinations of the anterior portion of the eye, the optic media, and the ocular fundus.

Haematology and clinical chemistry
Prior to initiation and at termination, blood samples from all rats were taken by puncture of the retro-orbital sinus after anesthetisation. These samples were submitted for haematological and clinical chemistry examination. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), thrombocytes, leucocytes and a blood smear examination (including differential white cell count).

For clinical chemistry analysis the following parameters were measured: Glucose, blood urea nitrogen, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, creatinine, creatinine kinase, calcium, phosphorous, sodium, potassium, chloride, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamic transferase.

Urinalysis
Urine samples were collected overnight from all rats prior to start of treatment and at termination. During urine collection, the rats were individually housed in metabolism cages and denied access to food. The following parameters were measured: Volume, pH, specific gravity, nitrites, blood, leukocytes, protein, glucose, ketones, bilirubin and urobilinogen.

Sacrifice and pathology
After 13 weeks of consecutive treatment, all surviving animals were sacrificed and subjected to a gross pathological examination. Any macroscopic findings were recorded.
The following organ weights were determined: Adrenals, brain (including brainstem), epididymides, kidneys, liver, spleen, seminal vesicles and testes or ovaries. Organ-to-body weight and organ-to-brain weights were determined.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aorta, bone marrow (sternum), brain, uterus/cervix/vagina, epididymides, oesophagus, eyes, femur (incl.

articular surface), heart, caecum, colon, rectum, duodenum, jejunum, ileum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal turbinate, nasal cavity, ovaries, pancreas, pituitary gland, prostrate, salivary glands (sublingual), spinal cord, sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroid, trachea and urinary bladder. All tissue sampled from the control and high dose group were examined histopathologically. From the mid- and low- dose only tissues from lungs, liver, kidneys and gross lesions were subjected to histopathological evaluation.

Statistics
Mean and standard deviation were calculated for all quantitative data. Comparisons with controls were done using ANOVA with a post hoc Dunnett´s t Test or Duncan´s multiple range test. A 95 % confidence level ($p < 0.05$) was used to determine statistically significant differences between control and treated groups.

II. RESULTS AND DISCUSSION

DIETARY ANALYSIS
The mixture was stable under ambient conditions for at least 10 days (within 8% of target concentration). Homogeneity was confirmed prior to study initiation (relative standard deviation (RSD) of 4-5%). One sample from the Week 8 20000 ppm Glyphosate mix exceeded the ± 10% of the target concentration (22200 ppm). One sample from the Week 12 6000 ppm Glyphosate mix exceeded the ± 10% of the target concentration (7300 ppm). Overall, for all diets tested for accuracy the RSD varied between 1% and 8%.

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
The only obvious treatment-related finding was diarrhoea observed in 10/10 males and 9/10 females in the high-dose group. Diarrhoea was also observed in one male of the low dose group.

Table 6.3.2-2: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Intergroup comparison of clinical findings

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Finding	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Diarrhoea	0/10	1/10	0/10	10/10	0/10	0/10	0/10	9/10

C. BODY WEIGHT
Mean body weights were not significantly different between test substance and control animals. The mean body weight gains were however significantly different from controls at several intervals (see table below). The total mean body weights over the entire study period were not statistically different.

Table 6.3.2-3: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Intergroup comparison of body weights and body weight gain – selected time points from start of study

Time point	Mean body weight or body weight gain [g]					
	Initial body weight	**Final body weight**	**Weight gain Day 43**	**Weight gain Day 50**	**Weight gain Day 85**	**Total weight gain**
Dose [ppm]	**Males**					
0	224.6	510.3	-3.4	39.4	12.6	285.7
2000	231.0	↑530.8	↑9.1	↓29.3	↓9.2	↑299.7
6000	229.0	↑526.0	↑**17.1***	↓28.6	↓11.1	↑297.0
20000	224.6	↑512.9	↑**21.1****	↓**19.3**** **(-51%)**	↓**1.9*** **(-85%)**	↑288.4
	Females					

Table 6.3.2-3: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted] 1993): Intergroup comparison of body weights and body weight gain – selected time points from start of study

Time point	Mean body weight or body weight gain [g]					
	Initial body weight	Final body weight	Weight gain Day 43	Weight gain Day 50	Weight gain Day 85	Total weight gain
0	178.3	321.9	6.9	16.6	6.1	143.7
2000	179.7	↑331.3	↑7.5	↓14.7	↑6.2	↑151.6
6000	176.0	↓316.1	↑7.8	↓15.7	↓2.4	↓140.1
20000	176.9	↓309.0	↑7.8	**↓4.8* (-71%)**	↓2.8 (-54%)	↓132.0

* Statistically significant from controls, $p < 0.05$ (Student´s t-test, 2-sided);
** Statistically significant from controls, $p < 0.01$ (Student´s t-test, 2-sided)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

There were no treatment-related effects. The mean food consumption in treatment groups was not significantly different from controls.

Calculated mean test compound intakes are presented in the following table.

Table 6.3.2-4: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Overall mean test compound intake [mg/kg bw/day]

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Achieved dose [mg/kg bw/day]	0	125.2	371.9	1262.1	0	156.3	481.2	1686.5

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group.

Blood clinical chemistry

At termination, low dose males had a significantly decreased mean potassium (K^+) concentration as compared to control animals. Since this finding was restricted to the low dose group, it was not considered to be treatment-related.

High dose males showed a significantly lower ALT value at termination than control males. However, because of the minor degree of change, the absence of a dose-response at the other dose levels this finding was considered to be of no biological relevance. The increased total bilirubin value in high-dose males at termination is also considered to be of no biological relevance.

It should be noted that blood chemistry was not carried out in some cases because the samples were inadvertently discarded.

Table 6.3.2-5: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Intergroup comparison of selected clinical chemistry parameters pre-dose and at termination

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
ALT [U/L]	48	↓35	↓41	**↓32***	42	↓39	↓35	↓30

Table 6.3.2-5: 90 Day Range Feeding Study of Glyphosate in Rats ([redacted] 1993): Intergroup comparison of selected clinical chemistry parameters pre-dose and at termination

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Total bilirubin [mg/dL]	0.3	0.3	↑0.5	↑**0.6***	0.2	↑0.3	↑0.3	↑0.3
Potassium [mmol/L]	6.9	↓**5.8****	↓6.2	↓6.8	5.8	↑6.2	↓5.7	↑6.2
Potassium [mmol/L] (pre-dose)	5.2	↓5.1	↓4.8	5.2	5.2	↓**4.6***	↓**4.4****	↓4.7

* Statistically significant from controls, $p < 0.05$;
** Statistically significant from controls, $p < 0.01$

G. URINALYSIS

At termination there were marginal increases in the mean scores for ketones, blood, protein and red blood cells (RBCs) in the mid- and high-dose males. The number of affected rats and their respective scores are shown in the Tables below. The lower protein value observed in high-dose males was statistically different from control, but still within the normal range. The presence of blood and RBCs is minimally elevated in all treated groups when compared to control animals. However, the observation of a few RBCs is common in male rats and this mild degree cannot be attributed unequivocally to the test substance. In mid- and high-dose females the number of rats with 1 RBC/high power microscope field was also increased. Together with the appearance of blood in these groups this change seems to be treatment-related. In addition, there were no indications for microscopic haematuria found during the histopathological examinations. Therefore, the changes in urine analysis parameters in the mid-dose group are considered not to be adverse effects.

Table 6.3.2-6: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Urinalysis at termination – group mean values for selected parameters

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Ketones [mg/dL]	0.0	0.0	**0.4***	0.1	0.0	0.0	0.0	0.0
Blood [mg/dL]	0.2	1.5	1.4	**2.1** (10.5 times higher)**	0.2	0.2	1.0	0.6 (3 times higher)
Protein [mg/dL]	1.2	0.8	1.1	**0.5***	0.1	0.4	0.3	0.0
RBC [cells/hpf]	0.3	0.7	0.8	**1.6****	0.2	0.2	**1.0****	0.6

* Statistically significant from controls, $p < 0.05$;
** Statistically significant from controls, $p < 0.01$

Table 6.3.2-7: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Urinalysis at termination – affected animals for selected parameters

Parameter	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Ketones (Score 0)	10/10	10/10	6/10	9/10	10/10	10/10	10/10	10/10
Ketones (Score 1)	0/10	0/10	4/10	1/10	0/10	0/10	0/10	0/10
Blood (score 0)	8/10	3/10	3/10	2/10	8/10	8/10	5/10	7/10
Blood (score 1)	2/10	3/10	3/10	3/10	2/10	2/10	2/10	0/10
Blood (score 2)	0/10	2/10	2/10	1/10	0/10	0/10	2/10	3/10
Blood (score 3)	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Blood (score 4)	0/10	2/10	1/10	4/10	0/10	0/10	1/10	0/10
Protein (score 0)	1/10	3/10	1/10	6/10	9/10	7/10	7/10	10/10

Table 6.3.2-7: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Urinalysis at termination – affected animals for selected parameters

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
Parameter	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Protein (score 1)	5/10	6/10	7/10	3/10	1/10	2/10	3/10	0/10
Protein (score 2)	4/10	1/10	2/10	1/10	0/10	1/10	0/10	0/10
RBC (score 1)	3/10	7/10	8/10	6/10	2/10	2/10	8/10	6/10
RBC (score 2)	-	-	-	1/10	-	-	1/10	-
RBC (score 3)	-	-	-	0/10	-	-	-	-
RBC (score 4)	-	-	-	2/10	-	-	-	-

- No finding

H. NECROPSY

Organ weights

The mean adrenal weights in high- and low-dose males were significantly decreased, whereas the relative adrenal weights were only significantly decreased in high dose males. In females of the high-dose group relative spleen weights were significantly increased when compared to controls.

Table 6.3.2-8: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Results from absolute and relative organ weight determination

	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
Mean adrenal weight [g]	0.070 ± 0.009	↓**0.059* ± 0.008**	↓0.061± 0.014	↓**0.052** ± 0.006 (-26%)**	0.093 ± 0.013	↓0.080± 0.008	↓0.086± 0.022	↓0.080± 0.010
Relative adrenal weight [% bw]	0.014 ± 0.003	↓0.012± 0.002	↓0.012± 0.003	↓**0.011** ± 0.001 (-21%)**	0.031 ± 0.004	↓0.026± 0.004	↓0.029± 0.008	↓0.028± 0.002
Spleen weight [g]	0.785 ± 0.103	↑0.786± 0.093	↑0.808± 0.121	↓0.752± 0.090	0.548 ± 0.071	↑0.598± 0.066	↑0.589± 0.103	↑0.648± 0.030 (+18%)
Relative spleen weight [% bw]	0.157 ± 0.026	↓0.154± 0.018	↑0.160± 0.024	↓0.152± 0.015	0.180 ± 0.017	↑0.192± 0.029	↑0.198± 0.026	↑**0.223** ± 0.035 (+24%)**

* Statistically significant from controls, $p < 0.05$;
** Statistically significant from controls, $p < 0.01$

Gross pathology

A few gross lesions were noted at necropsy in all dose groups. Swollen, reddened sublingual salivary glands were observed in one control male and in one treated male and female of some test substance groups (see table Table 6.3.2-). Therefore, this finding is considered not to be related to treatment.

Table 6.3.2-9: 90 Day Range Feeding Study of Glyphosate in Rats [redacted] 1993): Summary of necropsy findings

Finding	Dietary concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Sublingual salivary glands: enlarged and/or reddened	1/10	0/10	1/10	1/10	0/10	1/10	0/10	1/10

Histopathology
There were no histopathological findings related to treatment. The incidence of findings was low and of a type commonly found in rats of this strain and age.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. The only treatment-related clinical finding was diarrhoea in all high-dose males and in 9 high-dose females. Mean body weights were comparable between all groups. The mean body weight gains were significantly different between groups at several time points, but the total mean body weight gain was similar in all dose groups. There were no differences in food consumption. There were no treatment-related findings in the haematological and clinical chemistry parameters. Urinalysis showed marginal increases in the mean scores for ketones, blood, protein and red blood cell counts in mid- and high-dose males, which were of no biological relevance. In mid- and high-dose females, microscopy revealed an increased number of rats with 1 red blood cell per high power field. Together with the appearance of blood in these groups this change seems to be treatment-related. The gross changes noted at necropsy were few and inconclusive with respect to association with exposure to the test article. The most prevalent abnormality was the non-treatment related swollen, reddened sublingual salivary glands. The mean weight of the adrenal glands in the high and low dose male groups was significantly decreased. The group mean weight of the spleen was significantly increased for the high dose female group when the spleen weights were compared as a percent of body weight. Gross and microscopic examinations did not identify tissue changes which were related to treatment. These findings are in agreement with clinical chemistry, hematologic and gravimetric data collected during the conduct of this study.

The results indicate that under the conditions of this investigation, dietary levels of up to 20000 ppm glyphosate were tolerated without significant pathophysiologic alterations.

According to the study authors, the Maximum Tolerated Dose (MTD) was 20000 ppm glyphosate and the No Observable Adverse Effects Level (NOAEL) was reported to be 6000 ppm glyphosate.
The NOAEL is agreed by the RMS, but this is however based on the occurrence of diarrhoea in both sexes, decreased body weight gain at day 50 and 85 in both sexes, decreased absolute and relative adrenal weights in males, increased absolute and relative spleen weight in females and blood in urine at the LOAEL of 20000 ppm.

Assessment and conclusion by applicant:
In this sub-chronic toxicity study, groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0, 2000, 6000 or 20000 ppm glyphosate acid (equivalent to 0, 125.2, 371.9 or 1262.1 mg/kg bw/day for males and 0, 156.3, 481.2 or 1686.5 mg/kg bw/day for females) for 13 consecutive weeks.

The results indicate that under the conditions of this investigation, dietary levels of up to 20000 ppm glyphosate (equivalent to 1262.1 mg/kg bw/day for males and 1686.5 mg/kg bw/day for females) were tolerated without significant pathophysiologic alterations.

Based on the results of this study, the Maximum Tolerated Dose (MTD) was 20000 ppm glyphosate (equivalent to 1262.1 mg/kg bw/day for males and 1686.5 mg/kg bw/day for females).
There were no consistent effects at the dose levels of 6000 and 20000 ppm. At the highest dose of 20000 ppm there were statistically significant effects on organ weights, i.e. reduced adrenal weights in males and increased spleen weights in females. Therefore, the No Observable Adverse Effect Level (NOAEL) was set at 6000 ppm glyphosate (equivalent to 371.9 mg/kg bw/day for males and 481.2 mg/kg bw/day for females).

Assessment and conclusion by RMS:
The RMS considers this study as acceptable as the deviations from the current version are due to the fact that the study was aligned to an older version of OECD TG 408. The conclusions drawn by the applicant are supported by the RMS and the NOAEL of 6000 ppm glyphosate (equivalent to 371.9 mg/kg bw/day for males and 481.2 mg/kg bw/day for females) is agreed. According to the RMS, this NOAEL is based on the occurrence of diarrhoea in both sexes, decreased body weight gain at day 50 and 85 in both sexes, decreased absolute and relative adrenal weights in males, increased absolute and relative spleen weight in females and blood in urine at the LOAEL of 20000 ppm. The same NOAEL was also proposed in the previous assessment (RAR, 2015).

In the previous assessment in the RAR (2015), the following was concluded by the RMS DE:
" …. the study by [redacted] (1993; TOX9650149), former assessment as "acceptable" was changed by the RMS into "supplementary" because the batch number but no purity of the test material was given. "
However, the current RMS NL notes that the purity of the test substance is provided in the study rapport in an certificate of analysis. The same NOAEL of 6000 ppm was set based on diarrhoea in m/f; blood in urine; organ weight changes without pathological findings.

B.6.3.2.5. Oral 90-day toxicity study in rats – study 5

Data point:	CA 5.3.2/008
Report author	[redacted]
Report year	1992 (study report)
Report title	90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990)
Report No	[redacted].882.90 OR
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	The following organs were not noted in the gross pathology and histopathological evaluation: aorta, cervix, epididymides, mammary gland, peripheral nerve, prostate, skeletal muscle and bone, skin, spinal cord, thymus, vagina. The following organs were not weighed: testes, epididymides, prostate and seminal vesicles with coagulating glands, thymus, heart, brain, and spleen. Thyroid hormone levels (i.e., T4, T3, and TSH) were not measured. No ophthalmological examination and urinary analysis were conducted. Sensory reactivity to different stimuli was not evaluated. The rationale for dose selection was not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point:	CA 5.3.2/009
Report author	[redacted]
Report year	1992 (appendix to study report)
Report title	90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) (Appendix)
Report No	[redacted].882.90 OR
Document No	Not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point:	CA 5.3.2/010

Report author	[redacted]
Report year	June 1994 (1st amendment); November 1994 (2nd amendment)
Report title	Amendments to Final Report. 90–day Oral Toxicity Study in Wistar Rats
Report No	[redacted].882.90 OR
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable but with restrictions (reliable with restrictions) due to limited histopathology and organ weight reporting (refer to deviations above).

Executive Summary

In a 90–day toxicity study, groups of 10 male and 10 female Wistar rats were administered technical glyphosate for 90 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm. These dose levels were equivalent to 0, 14.0, 147.3, or 1358.6 mg/kg bw/day for males and 0, 18.6, 195.7, or 2012.4 mg/kg bw/day for females, respectively. A high-dose recovery group was administered 20000 ppm (equivalent to 1482.6 mg/bw/day for males and 1878.3 mg/kg bw/day for females) for 13 weeks and then were followed for 4 weeks without treatment before being sacrificed.

General clinical observations were done daily. Body weights and food consumption were assessed in weekly intervals. Haematological and blood biochemistry parameters were evaluated at sacrifice. At the end of the scheduled period, the animals were killed and subjected to a post-mortem examination and selected organs were weighed and tissues were taken for subsequent histopathology examination.

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. While significant changes were observed in body weight gains, haematology parameters, and clinical chemistry parameters, these changes were considered to be incidental in nature. At 20000 ppm, decreased body weight gains were observed in female rats; body weight changes were not observed in males. Significant changes were observed in haematology values for males (decreased eosinophils at the high dose, increased mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration at the mid dose and high-dose recovery group, decreased red blood cells at the mid and low dose, and decreased haematocrit at the low dose). There were no significant changes to haematology parameters for female rats at any dose level.

With regards to clinical chemistry parameters, there was an increase in alkaline phosphatase activity (high dose) and a decrease in albumin levels (low dose) and total bilirubin (high-dose recovery group) in males. For females, there was a decrease in creatinine (all dose levels) and calcium concentrations (all doses except the recovery group), while there was an increase in glucose levels (high dose). There also was decrease in glucose levels for the high-dose recovery group when compared with the high dose.

There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

The conclusions drawn are supported by the RMS and the NOAEL of 2000 ppm glyphosate (corresponding to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females) is agreed. According to the RMS, this NOAEL is based on decreased body weight in females, increased alkaline phosphatase in males and increased blood glucose in females at the LOAEL of 20000 ppm.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Technical Glyphosate
Description:	Solid, odourless white coloured crystals
Lot/Batch#:	FSG 03090 H/05 March 1990
Purity:	96.8%
Stability of test compound:	Fairly stable for 30 days under ambient temperature and stored in polyethylene lined stainless steel drums

2. **Vehicle and/ or positive control:** Plain diet / none

3. **Test animals:**

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	8 weeks
Sex:	Male and female
Weight at dosing:	♂ group means 163 ± 17.1 g; ♀ group means 132 ± 14.6 g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold Mohur" brand powdered rat feed, *ad libitum*,
Water:	Deep borewell water passed through activation charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Groups of 3-5 rats/ sex in steam sterilized standard polypropylene rat cages with stainless steel top grill
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 40 – 70% Air changes: 10 - 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: May 1991 to September 1991

Animal assignment and treatment:
Groups of ten male and ten female Wistar rats were administered technical glyphosate for 90 consecutive days via the diet at concentrations calculated to achieve dose levels of 0 (control), 20, 2000, or 20000 ppm.

Table 6.3.2-1: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) ([redacted], 1992): Animal assignment

Test group	Dose Level (ppm)	Males	Females
Control	0	10	10
Low	200	10	10
Intermediate	2000	10	10
High	20000	10	10
High Recovery	20000	10	10

The required amount of finely ground test compound was weighed and mixed manually with 1.0 kg powdered rat feed to prepare the premix. The premix was added in portions to the remaining quantity of feed and mixed. To the control group feed, the powdered rat feed was mixed for 20 minutes. Prepared feed was sampled at different levels for assaying the test compound concentration.

All test groups received diet specifically prepared for the group ad libitum. Animals were trea ted for seven days a week for thirteen weeks. The recovery group received powdered normal rat feed for four weeks following thirteen weeks of treatment.

Mortality
Each animal was checked for mortality or signs of morbidity once daily during the treatment period.

Clinical observations
General clinical observations were done once daily. Cage side observations included changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern.

Body weight
Individual body weights were recorded at the end of each week.

Food consumption and utilisation
Daily feed consumption per cage measured during the last two days of the week.

Ophthalmoscopic examination
Dedicated eye examinations were not performed.

Haematology and clinical chemistry
One day prior to sacrifice, blood smears from surviving rats were made by tail clipping and the blood smears were stained by Wright's stain. At the end of the study all the surviving animals were fasted overnight (water allowed) and blood was collected from abdominal aorta under ether anaesthesia.

For haematology, differential leucocyte counts were done manually. Fractions of blood were taken for coagulation time. For haematology and plasma separation, blood was heparinized. The following haematological parameters were measured: white blood cell (WBC) count, red blood cell (RCB) count, haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

For clinical chemistry analysis the following parameters were measured: glucose (GLU), total bilirubin (TOT BIL), creatinine (CREAT), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), enzymatic blood urea nitrogen, albumin (ALB), alkaline phosphatase (ALP), total protein, sodium, potassium, and calcium (Ca^{2+}).

Urinalysis
Not performed.

Sacrifice and pathology
At the end of the study, rats were fasted overnight and sacrificed by total blood collection under either anaesthesia. After sacrifice, a detailed gross necropsy was conducted. The following organs were collected and weighted from every animal: adrenals (both), gonads (both), kidneys (both), and liver. The relative organ weights were determined as a percentage of body weight. Tissue samples from brain, lungs, heart, eye (Bouin's fluid), spleen, lymph nodes (mesenteric), oesophagus, stomach, small and large intestines, salivary gland, liver, pancreas, kidneys (both), urinary bladder, testes/ovaries (both), seminal vesicles/uterus, pituitary, thyroid and adrenals (both) were preserved in neutral buffered formalin and 5 µm sections were stained. The tissues sec tions were studied for histopathological changes. All the tissues from all animals in control and high-dose group and all lesions were subjected to detailed histopathological studies.

Statistics
Body weight and feed consumption were compared using the Bartlett's test for homogeneity of variance and one-way classification analysis of variance (ANOVA) and Dunnet's multiple pairwise comparison. The clinical laboratory analysis data and organ weight data from the control group were compared with the data of t reated groups by the Bartlett's test for homogeneity of variance followed by ANOVA, where the variances proved to be heterogeneous, the data was transformed using the appropriate transformation. If ANOVA of homogeneous data was significant, Dunnet's pairwise comparison procedure was used to compare the treated group with the control group individually. All analyses were evaluated at 5 % probability level.

II. RESULTS AND DISCUSSION

DIETARY ANALYSIS
Five batches of diet mixes were analysed for test compound concentration and were found to contain a mean concentration of 0, 194 ± 7.2, 1982 ± 42, 19706 ± 373 ppm of Glyphosate in experimental diet prepared for the four dose groups as against nominal concentration of 0, 200, 2000 and 20000 ppm respectively.

A. MORTALITY
There was no mortality in any of the study groups during treatment or recovery period.

B. CLINICAL OBSERVATIONS
In general there were no clinical signs of toxicity observed in any of the treatment groups. However, respiratory effects (i.e., nasal discharge) was reported in treatment groups; this effect appeared to reduce during the recovery period.

C. BODY WEIGHT
For males, body weights were comparable between treatment groups and control animals and there were no statistically significant differences. For females, initial body weights (week 0) were lower in treated than control groups and in the low-dose group this achieved statistical significance; however, body weights of low-dose animals were comparable to control animals in later weeks. High-dose animals also showed significantly lower body weights when compared to control animals from Week 3 through Week 6, as well as week 10. Similar results were not observed in the high-dose recovery group, which casts some doubt on the significance of these high dose changes. Results are presented in the following table:

Table 6.3.2-2: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Intergroup comparison of mean body weights

Body weights [g ± standard deviation]	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Week 0	166 ± 11.5	154 ± 22.0	163 ± 21.6	168 ± 22.8	166 ± 7.8	144 ± 18.1	**121 ± 15.0*** ↓	132 ± 8.6	129 ± 13.8	136 ± 17.5
Week 1	194 ± 14.3	185 ± 23.5	183 ± 22.6	194 ± 22.9	192 ± 9.3	152 ± 17.2	140 ± 12.6	142 ± 7.8	140 ± 13.7	152 ± 18.9
Week 2	223 ± 17.2	215 ± 25.4	209 ± 23.2	221 ± 26.6	218 ± 14.3	165 ± 16.6	153 ± 14.0	155 ± 6.3	150 ± 12.5	171 ± 23.8
Week 3	245 ± 17.4	234 ± 21.5	238 ± 25.9	237 ± 26.5	232 ± 17.8	178 ± 16.4	171 ± 13.3	166 ± 7.4	**161 ± 13.1* (-10%)**	181 ± 23.9
Week 4	256 ± 18.4	242 ± 20.9	247 ± 23.7	246 ± 26.5	252 ± 15.7	178 ± 16.5	179 ± 14.5	173 ± 6.4	165 ± 11.9	186 ± 24.9
Week 5	256 ± 17.2	257 ± 19.7	260 ± 25.5	260 ± 27.2	262 ± 15.8	181 ± 16.1	179 ± 16.4	173 ± 7.0	**164 ± 14.1* (-9%)**	191 ± 31.6
Week 6	274 ± 18.9	268 ± 22.7	266 ± 24.2	266 ± 27.8	268 ± 17.7	187 ± 16.6	182 ± 13.1	177 ± 8.3	**168 ± 16.1* (-10%)**	193 ± 31.1
Week 7	295 ± 17.9	280 ± 24.0	284 ± 23.2	282 ± 29.0	286 ± 20.7	196 ± 19.1	193 ± 16.9	185 ± 8.6	181 ± 22.6	200 ± 29.3

Table 6.3.2-2: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Intergroup comparison of mean body weights

Body weights [g ± standard deviation]	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Week 8	300 ± 18.5	284 ± 25.4	290 ± 22.4	287 ± 29.4	288 ± 19.2	202 ± 17.3	194 ± 18.6	189 ± 8.2	186 ± 24.7	203 ± 26.1
Week 9	307 ± 20.6	292 ± 26.3	294 ± 20.4	295 ± 32.0	292 ± 20.6	204 ± 15.5	197 ± 16.7	192 ± 6.8	185 ± 21.9	202 ± 25.2
Week 10	313 ± 19.3	300 ± 28.5	297 ± 21.0	302 ± 33.7	302 ± 20.3	212 ± 14.7	204 ± 19.9	198 ± 8.8	**184 ± 22.2*(-13%)**	205 ± 26.5
Week 11	321 ± 21.7	300 ± 30.5	297 ± 15.3	301 ± 32.2	304 ± 19.8	212 ± 15.6	203 ± 19.6	199 ± 6.9	189 ± 22.5	204 ± 25.1
Week 12	321 ± 24.7	306 ± 28.8	305 ± 19.6	307 ± 30.4	310 ± 27.1	213 ± 16.3	205 ± 21.4	205 ± 8.4	194 ± 22.8	202 ± 24.1
Week 13	322 ± 23.2	315 ± 24.1	313 ± 20.2	313 ± 36.5	315 ± 30.7	215 ± 16.4	205 ± 22.2	208 ± 10.6	199 ± 21.7	209 ± 20.5

* Significant at P = 0.05 over control group value
↓ Decreased

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

All groups receiving Glyphosate performed similarly to their respective controls. Test compound intakes are presented in the table below. The RMS notes that the dietary intake of the 20000 ppm recovery group was not reported in the study report and could therefore not be confirmed.

Table 6.3.2-3: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Test compound intake

	Mean dietary concentration of glyphosate [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Dose [mg/kg bw/day]	0	14.0	147.3	1358.6	1482.6	0	18.6	195.7	2012.4	1878.3

E. OPHTHALMOSCOPIC EXAMINATION

Ophthalmoscopic eye examinations were not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no major treatment-related changes apparent in both sexes. Male rats showed incidental significant changes, including decreased eosinophils at the high dose, increased MCH and MCHC in the mid and high dose recovery groups, decreased RBC at the mid and low dose, and decreased HCT at the low dose. There were no significant changes to haematology parameters for female rats at any dose level.

Table 6.3.2-4: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted], 1992): Intergroup comparison of selected haematology parameters (mean ± SD) in male rats

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
RBC [10^6/mm^3]	9.26 ± 0.36	**8.72 ± 0.37*↓**	**8.38 ± 0.59*↓**	8.96 ± 0.47	8.48 ± 0.69
HCT [%]	37.9 ± 1.97	35.1 ± 1.87	35.7 ± 2.24	37.6 ± 3.12	39.6 ± 1.74
MCH pg [10^{-12}]	11.9 ± 0.57	**13.6 ± 1.32*↑**	**13.1 ± 0.93*↑**	12.7 ± 1.01	**18.9 ± 1.34**↑**
MCHC [%]	29.2 ± 2.09	**34.1 ± 3.89*↑**	30.9 ± 1.67	30.5 ± 3.06	**39.7 ± 2.24**↑**
Eosinophils [%]	3 ± 1.49	2 ± 2.22	2 ± 1.83	1 ± 1.05	2 ± 2.84

* Significant at P = 0.05 over control group value
** Significant difference at P = 0.05 between the high-dose group and the high-dose recovery group
↓ Decreased; ↑ Increased

Blood clinical chemistry
For males, there was a significant increase in ALP activity at the high dose that remained high (although not statistically significant) even at the end of the recovery period. Low-dose males exhibited a significant decrease in albumin levels. High-dose recovery males exhibited a significant decrease in total bilirubin.

For females, a significant decrease in creatinine concentrations was observed at all dose levels. Calcium concentrations were low in all treatment groups except the high-dose recovery group that showed levels that were similar to control animals. Decreases in calcium concentrations reached significance at the mid dose, with the low- and high-dose groups showing a non-significant decrease. A significant increase in glucose levels also were observed at the high dose, while a significant decrease in glucose levels was observed in the high-dose recovery group when compared with the high dose.

Table 6.3.2-5: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Intergroup comparison of selected clinical chemistry parameters (mean ± SD) in male rats

Parameter	Males				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
ALP [IU/L]	105 ± 32.1	121 ± 31.1	106 ± 29.5	**157 ± 45.7*↑ (+50%)**	150 ± 55.7
ALB [g/dL]	3.4 ± 0.11	**3.3 ± 0.09*↓**	3.3 ± 0.16	3.4 ± 0.08	3.5 ± 0.18
TOT BIL [mg/dL]	0.51 ± 0.20	0.45 ± 0.20	0.47 ± 0.22	0.71 ± 0.23	**0.35 ± 0.10**↓**

* Significant at P = 0.05 over control group value
** Significant difference at P = 0.05 between the high-dose group and the high-dose recovery group
↓ Decreased; ↑ Increased

Table 6.3.2-6: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Intergroup comparison of selected clinical chemistry parameters (mean ± SD) in female rats

Parameter	Females				
	Dose level [ppm]				
	0	200	2000	20000	20000 (recovery group)
GLU [mg/dL]	114 ± 25.1	124 ± 16.0	124 ± 20.3	**141 ± 20.6*↑ (+24%)**	**110 ± 13.3**↓**
CREAT [mg/dL]	0.63 ± 0.06	**0.55 ± 0.06*↓**	**0.56 ± 0.04*↓**	**0.56 ± 0.03*↓**	**0.50 ± 0.06*↓**
Ca^{2+} [mg/dL]	10.0 ± 0.65	9.4 ± 0.51	**9.2 ± 0.48*↓**	9.6 ± 0.50	10.2 ± 1.05

* Significant at P = 0.05 over control group value
** Significant difference at P = 0.05 between the high-dose group and the high-dose recovery group
↓ Decreased; ↑ Increased

G. URINALYSIS
Not performed

H. NECROPSY
Organ weights
There were no intergroup differences in either sex.

Gross pathology
Gross pathological observations were in general evenly distributed across all treatment groups. A few gross lesions were observed in the liver and lung; however, these changes were not considered treatment related.

Table 6.3.2-7: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Summary incidence of gross pathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Liver										
Focal thickening of capsule	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Yellow discoloura-tion of papillary process	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10
Pale	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	2/10	0/10
Lungs										
Petechiae	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Table 6.3.2-7: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Summary incidence of gross pathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Consolidation	0/10	0/10	0/10	0/10	2/10	1/10	0/10	0/10	0/10	0/10
Emphysema	0/10	0/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10
Echymoses	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10

Histopathology
There were no treatment-related findings reported during the histopathological evaluation. Hyperplasia of the lymphoid nodules in the submucosa of the duodenum, ileum and colon were observed but these effects were considered to be spontaneous lesions commonly observed in Wistar rats. An instance of focal chronic hepatitis and interstitial lymphoid cell infiltration in the prostate also appeared to be incidental in nature.

Table 6.3.2-8: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted], 1992): Summary incidence of histopathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Liver										
Inflammation, chronic	0/10	---	---	1/10	---	0/10	---	---	0/10	---
Bile duct proliferation	3/10	---	---	2/10	---	0/10	---	---	0/10	---
Lungs										
Congestion	8/10	---	---	9/10	1/2	7/10	---	---	6/10	---
Lymphoid hyperplasia	2/10	---	---	4/10	1/2	6/10	---	---	2/10	---
Atelectasis	7/10	---	---	9/10	1/2	7/10	---	---	5/10	---

Table 6.3.2-8: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) ([redacted] 1992): Summary incidence of histopathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Broncho, pneumonia	0/10	---	---	0/10	2/2	1/10	---	---	0/10	---
Emphysema, alveolar	2/10	---	---	0/10	1/2	2/10	---	---	0/10	---
Perivascular lymphocytic aggregation	4/10	---	---	4/10	0/2	3/10	---	---	3/10	---
Kidneys										
Lymphocytic infiltration	1/10	---	1/1	2/10	---	2/10	---	---	0/10	---
Hydro-nephrosis	0/10	---	1/1	0/10	---	0/10	---	---	0/10	---
Duodenum										
Inflammation, acute	0/10	1/?	---	0/10	---	0/10	---	---	0/10	---
Lymphoid hyperplasia	0/10	0/?	---	0/10	---	1/10	---	---	1/10	---
Ileum										
Inflammation, acute	0/10	1/2	---	0/10	---	0/10	---	---	0/10	---
Lymphoid hyperplasia	1/10	1/2	---	2/10	---	0/10	---	---	0/10	---
Colon										
Lymphoid hyperplasia	2/10	---	---	2/10	---	2/10	---	---	1/10	---
Mesenteric Lymph Nodes										
Lymphoid hyperplasia	0/10	1/1	---	0/10	1/1	0/10	---	---	0/10	---
Trachea										
Lymphocytic infiltration	5/10	---	---	3/10	---	3/10	---	---	3/10	---

Table 6.3.2-8: 90–day Oral Toxicity Study with Glyphosate Technical in Wistar Rats (FSG 03090 H/05 March 1990) [redacted] 1992): Summary incidence of histopathological findings

Findings	Dose level [ppm]									
	Males					Females				
	0	200	2000	20000	20000 (recovery group)	0	200	2000	20000	20000 (recovery group)
Lymphoid hyperplasia	0/10	---	---	0/10	---	1/10	---	---	0/10	---

III. CONCLUSIONS

There was no mortality in any of the study groups during treatment or recovery period. In general, there were no clinical signs of toxicity observed in any of the treatment groups. While significant changes were observed in body weight gains, haematology parameters, and clinical chemistry parameters, these changes were considered to be incidental in nature. At 20000 ppm, decreased body weight gains were observed in female rats; body weight changes were not observed in males. Significant changes were observed in haematology values for males (decreased eosinophils at the high dose, increased mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration at the mid dose and high-dose recovery group, decreased red blood cells at the mid and low dose, and decreased haematocrit at the low dose). There were no significant changes to haematology parameters for female rats at any dose level.

With regards to clinical chemistry parameters, there was an increase in alkaline phosphatase activity (high dose) and a decrease in albumin levels (low dose) and total bilirubin (high-dose recovery group) in males. For females, there was a decrease in creatinine (all dose levels) and calcium concentrations (all doses except the recovery group), while there was an increase in glucose levels (high dose). There also was decrease in glucose levels for the high-dose recovery group when compared with the high dose.

There were no notable intergroup differences in organ weights. No gross pathology or histopathology findings attributed to administration of glyphosate were recorded.

According to the 2nd amendment to study report created in November of 1994, the No Observed Effect Level (NOEL) was determined to be 2000 ppm in diet, which is equivalent to 147.3, 195.7, and 171.5 mg/kg bw/day for male, female and combined sex, respectively.

Assessment and conclusion by applicant:
In this 90–day toxicity study, groups of 10 male and 10 female Wistar rats were administered technical glyphosate for 90 consecutive days via the diet at concentrations of 0, 200, 2000, or 20000 ppm. These dose levels were equivalent to 14.0, 147.3, or 1358.6 mg/kg bw/day for males and 18.6, 195.7, or 2012.4 mg/kg bw/day for females, respectively. A high-dose recovery group was administered 20000 ppm (equivalent to 1482.6 mg/bw/day for males and 1878.3 mg/kg bw/day for females) for 13 weeks and then was followed for 4 weeks without treatment before being sacrificed.
Dosing Wistar rats via the diet with glyphosate produced decreased body weight gains in high-dose females. Additionally, there were significant increases in ALP activity in male rats at the high-dose level, as well as significant decreases in creatinine and calcium levels in female rats. The latter effects (creatinine and calcium) did not show a dose-response relationship and were therefore considered to be incidental. Female rats also exhibited increased glucose levels at the high-dose level. There were no treatment-related effects at any dose level with regards to mortality, clinical signs of toxicity, haematology, organ weight and gross and histopathological findings. Based on this information, the no adverse observed effect level is determined to be 2000 ppm in diet corresponding to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females.

Assessment and conclusion by RMS:

The RMS considers this study as acceptable but with restrictions (reliable with restrictions) due to limited histopathology and organ weight reporting (refer to deviations). The conclusions drawn are supported by the RMS and the NOAEL of 2000 ppm glyphosate (corresponding to 147.3 mg/kg bw/day for males and 195.7 mg/kg bw/day for females) is agreed. According to the RMS, this NOAEL is based on decreased body weight in females, increased alkaline phosphatase in males and increased blood glucose in females at the LOAEL of 20000 ppm. The same NOAEL on the same basis was proposed in the previous assessment (RAR, 2015).

However, in the previous assessment in the RAR (2015), the following was concluded on the status of the study by the RMS DE: *"[……] former assessment as "acceptable" was changed by the RMS into "supplementary" [.....]. This same holds true for a study by* [redacted] *(1992, TOX9551096) in which reporting deficiencies were noted. Mean dietary intake was calculated in an amendment but not for the recovery group and no information was given which of the salivary gland had been examined histologically. Apparently, no additional control group was employed for the recovery part. "*
The current RMS disagrees and considers this study as acceptable but with restrictions (reliable with restrictions) as the dietary intake for the high dose recovery group is not relevant for NOAEL setting and in the study report it is shown that histopathological examination of the salivary gland has been performed.

B.6.3.2.6. Oral 90-day toxicity study in rats – study 6

Data point	CA 5.3.2/011
Report author	[redacted]
Report year	1991
Report title	Glyphosate – 13 week dietary toxicity study in rats
Report No	7136
Document No	Not reported
Guidelines followed in study	FIFRA 82-1; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining HDL, LDL, T4, T3 and TSH; organ weights of the thyroid gland was not determined; histopathology was performed without bone/bone marrow, cervix, coagulating glands, spinal cord and vagina. Vaginal smears not taken. Sensory reactivity to different stimuli was not evaluated. In addition, individual data for weekly body weights, food consumption, water consumption are not reported. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary
This study was designed to give toxicity information over 13 weeks on Glyphosate administered to rats via the diet. The concentrations of the diet were adjusted weekly to achieve dose levels of 0, 30, 300 or 1000 mg/kg bw/day. The group size was 10 animals per sex and dose group.

The animals were examined for mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology.

There were no mortalities or clinical signs. Body weight, food intake, water consumption, ophthalmoscopy and haematology were unaffected by treatment. Glucose, total protein, albumin and creatinine were slightly and equivocally increased in high dose females. There was a reduction of urinary pH in high dose males. Gross pathology revealed no changes attributable to glyphosate treatment however an increased incidence and severity of "cellular alteration" (deep basophilic staining and enlargement of cytoplasm) in the parotid salivary gland of was observed in both sexes at 1000 mg/kg bw/day and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes.

The applicant proposed a NOAEL of 300 mg/kg bw/day. However, the RMS proposes a LOAEL of 30 mg/kg bw/day based on an increase in (very mild) cellular alterations in the parotid gland (refer to RMS comments below the study summary).

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:	Not reported

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	ca 5 – 6 weeks
Sex:	Male and female
Weight at dosing:	♂ 95 – 97 g; ♀ 67 – 68 g
Acclimation period:	9 days
Diet/Food:	SDS Rat and Mouse No. 1 Expanded (Fine Ground) Diet
Water:	Tap water, *ad libitum*
Housing:	2 of one sex per cage in suspended polypropylene cages (42.0 × 27.0 × 20.0 cm) with 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: 1989-06-22 to 1989-09-21/22

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 10 Sprague-Dawley rats per sex for a minimum of 90 days. Dietary concentrations were 0, 30, 300 and 1000 mg/kg bw/day.

Table 6.3.2-1: Glyphosate – 13 week dietary toxicity study in rats [redacted] 1991): Study design

Test group	Dietary concentration [mg/kg bw/day]	Males	Females
Control	0	10	10
Low	30	10	10
Mid	300	10	10
High	1000	10	10

Analysis of the test diet
A 100 g sample of diet from each group/sex was retained immediately after each diet preparation. In addition, usually 3 x 100 g samples were also taken for routine homogeneity and accuracy assessment from diets prepared for Weeks 1, 6 and 13. In addition, data proving homogeneity and 21-day stability of glyphosate were generated prior to the commencement of the study.

Mortality
Viability was checked once each morning and once as late as practicable each day.

Clinical observations
All animals were examined for reaction to treatment during the day. The onset, intensity and duration of these signs were recorded. All animals received a detailed clinical examination once each week.

Body weight
The weight of each animal was recorded once during the week before the start of treatment and once each week thereafter.

Food consumption and water consumption
The quantity of food consumed by each cage of animals was recorded once each week, commencing one week before the start of treatment and once each week thereafter.
Water consumption was measured gravimetrically on a weekly basis commencing one week before the start of treatment until the end of the study.

Ophthalmoscopy examination
The eyes of all animals in the Control and High dose groups were examined using an indirect ophthalmoscope after the application of a mydriatic agent (1 % Mydriacyl). Anterior, lenticular and fundic areas were evaluated. This ophthalmoscopic examination was undertaken pretrial and during Week 13 of treatment.

Haematology and clinical chemistry
Samples were taken from all rats from each group during Week 13 of dosing. Blood samples were collected from the orbital sinus under light ether anaesthesia.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total white blood cell count, differential white blood cell count and prothrombin time (Hepato Quick).

Clinical chemistry
The following parameters were determined: Alkaline phosphatase (ALP), aspartate amino transferase (AST), Alanine aminotransferase (ALT), creatinine (Crea), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), albumin/globulin ratio (AG-R), glucose (Glu), total cholesterol (Chol), total bilirubin (T.Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P), plasma cholinesterase, RBC cholinesterase and brain cholinesterase (ca. 0.5 g of brain was removed from each animal at necropsy and deep frozen (-20 °C) until assayed).

Urinalysis

Urine samples were collected from animals in metabolism cages. The samples were collected over a 4 h period of food and water deprivation. The following parameters were measured: Specific gravity, glucose, ketones, blood pigments, pH, protein, volume, bilirubin and urobilinogen.

Sacrifice and pathology
All animals were killed and necropsied. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissection and necropsy were performed under the supervision of a pathologist.

Organ weights
The following organs were weighed: Adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), pituitary, prostate, salivary glands (submaxillary, parotid and sublingual), spleen, testes (with epididymides), thymus and uterus.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals. Only one eye was processed and examined. In addition all other animals received histopathological examination of liver, kidneys and lungs.

Examination of parotid salivary glands was subsequently extended to include all Low and Intermediate dose group males and females.

The following tissues were investigated: Adrenals, aortic arch, bladder, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), kidneys, liver, lungs (perfused), mammary gland, mesenteric lymph node, muscle, oesophagus, ovaries (with fallopian tubes), pancreas, parotid salivary gland, pituitary, prostate, sciatic nerve, seminal vesicles, skin, spleen, stomach, sublingual salivary gland, submaxillary salivary gland, submandibular lymph node, testes (with epididymides), thymus, thyroid/parathyroid, tongue, trachea and uterus.

Statistics
Haematology, clinical chemistry, organ weight and body 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 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 in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used. Organ weights were also analysed conditional on body weight (i.e. analysis of covariance). Histopathology data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS

The group mean achieved dosages were generally in close agreement (100-103%) with the nominal values (30 ± 2, 304 ± 19 and 1011 ± 73 mg/kg bw/day in males and 31 ± 3, 300 ± 30 and 1020 ± 66 mg/kg bw/day in females in the 30, 300 and 1000 mg/kg bw/day dose groups, respectively). The majority of diets prepared for Weeks 1, 6 and 13 were seen to be within acceptable limits (± 10 %) for accuracy of concentration and homogeneity. In Week 1, the concentration for Intermediate Dose Group (females) was -11.0 % and the coefficient of variation was 25.4 %. A repeat analysis for this group from diets prepared for Week 2 showed an acceptable level of concentration (-0.7 %) and coefficient of variation (4.2 %). At Week 13 the concentration and coefficient of variation for Low Dose Group (males) were -12.5 % and 21.6 % respectively. No repeat analysis was carried out.

B. MORTALITY
There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS
There were no clinical signs in the control and treated groups that were considered to be due to administration of Glyphosate.

D. BODY WEIGHT

There were no notable intergroup differences in either sex.

E. FOOD CONSUMPTION AND WATER CONSUMPTION

There were no notable intergroup differences in total food or water consumed in either sex at any time.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

The only finding which showed statistical significance was an increase (300 %) in eosinophils in the male Intermediate dose group. This increase was not reflected in other WBC parameters and due to the lack of an effect in the High dose group was considered to be a chance effect. No notable intergroup differences were found for females.

Table 6.3.2-2: Glyphosate – 13 week dietary toxicity study in rats [redacted] 1991): Selected haematological findings (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Differential white blood cell count – Eosinophils [$\times 10^9$/L]	0.05 ± 0.07	↓0.04 ± 0.10	↑**0.20** ± 0.14**	↑0.10 ± 0.11	0.06 ± 0.08	↓0.02 ± 0.04	↓0.02 ± 0.04	↑0.07 ± 0.07

****** Statistically significant compared to control ($p \leq 0.01$)

Blood clinical chemistry

Alkaline phosphatase was increased (28%) in the male Intermediate dose group but this was considered to be a chance effect due to the lack of an effect in the High dose group and the high degree of variation seen in the individual values. The female High dose group showed slight increases in glucose (11%), total protein (9%), albumin (9%) and creatinine (8%). There were no other notable intergroup differences.

Table 6.3.2-3: Glyphosate – 13 week dietary toxicity study in rats [redacted] 1991): Selected clinical chemistry findings (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Alkaline phosphatase [iu/L]	317 ± 88	↓299 ± 57	↑**405* ± 102**	↑361 ± 48	222 ± 57	↑242 ± 72	↑255 ± 96	↑391 ± 100
Glucose [mmol/L]	7.36 ± 0.75	↓6.94 ± 0.68	↓6.85 ± 0.98	↓6.92 ± 0.93	7.29 ± 0.67	↑7.42 ± 0.50	↓7.14 ± 0.69	↑**8.10* ± 0 91 (+11%)**
Total protein [g/L]	67 ± 3	↓65 ± 1	67 ± 4	↓65 ± 3	67 ± 3	↑68 ± 3	↑69 ± 3	↑**73*** ± 3**
Albumin [g/L]	32 ± 1	32 ± 1	32 ± 2	32 ± 1	35 ± 2	35 ± 2	↑36 ± 2	↑**38* ± 2**
Creatinine [µmol/L]	48 ± 3	↓47 ± 3	↓47 ± 2	↓47 ± 2	52 ± 2	↑53 ± 2	↑53 ± 2	↑**56** ± 3**
Brain cholinesterase activity [iu/g]	13707 ± 2809	↑**17615** ± 3098**	↑**16527* ± 2455**	↑14715 ± 3533	15837 ± 4021	↓15388 ± 2955	↓13213 ± 3812	↓13588 ± 3266

* Statistically significant compared to control ($p \leq 0.05$);
** Statistically significant compared to control ($p \leq 0.01$);
*** Statistically significant compared to control ($p \leq 0.001$)

H. URINALYSIS

The pH was reduced in the male High dose group when compared with Controls. There were no notable intergroup differences for females.

Table 6.3.2-4: Glyphosate – 13 week dietary toxicity study in rats [redacted] 1991): Selected urinalysis findings (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
pH	8.8 ± 0.4	↓8.3 ± 0.8	↓8.6 ± 0.6	↓6.3 ± 0.7	8.1 ± 0.9	↑8.5 ± 0.5	↑8.2 ± 0.6	↓7.1 ± 1.1 (-12%)

I. NECROPSY

Organ weights
There were no notable intergroup differences in either sex.

Table 6.3.2-5: Glyphosate – 13 week dietary toxicity study in rats ([redacted] 1991): Selected absolute organ weights (week 13)

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Salivary gland [g]	1.412 ± 0.153	1.325 ± 0.127	1.403 ± 0.443	1.448 ± 0.299	0.846 ± 0.091	0.843 ± 0.118	0.831 ± 0.108	0.872 ± 0.187

Gross pathology
There were no findings which could be related to treatment with glyphosate.

Histopathology
The most notable findings were seen in the parotid salivary glands, where there was an increase in the incidence and severity of 'cellular alteration' (deep basophilic staining and enlargement of cytoplasm) in both sexes at 1000 and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes.

The incidence was statistically significant in both sexes of the intermediate and high dose groups and in the female low dose group. The finding was present in 7/10 male and 8/10 female Low dose group rats and 10/10 male and 9/10 female Intermediate and High dose rats. Three male Control rats and 2 female Control rats also had the finding (graded as very mild). The severity of the lesion tended to increase with increasing dose of glyphosate and in High dose males 5/10 animals affected were graded as severe, which reached statistical significance at the $p \leq 0.05$ level.

There were also a number of background changes usually seen in rats of this age and strain at the performing laboratory, considered to be unrelated to treatment with glyphosate.

Table 6.3.2-6: Glyphosate – 13 week dietary toxicity study in rats [redacted] 1991): Selected histopathological findings

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
Salivary gland	No abnormalities detected	7	3	**0****	**0****	8	**2***	**1****	**1****
	Parotid: cellular alteration (very mild)	3	7	6	0	2	7	7	1
	Parotid: cellular alteration (mild)	0	0	3	2	0	1	2	4
	Parotid: cellular alteration (moderate)	0	0	1	3	0	0	0	3

Table 6.3.2-6: Glyphosate – 13 week dietary toxicity study in rats (1991): Selected histopathological findings

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
	Parotid: cellular alteration (severe)	0	0	0	**5***	0	0	0	1
	Total incidence of finding	3	7	**10****	**10****	2	**8****	**9****	**9****
Mammary gland	No abnormalities detected	7	-	-	8	10	-	-	9
	Secretion present	3	-	-	2	0	-	-	0
	Adenocarcinoma [M]	0	-	-	0	0	-	-	1

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

III. CONCLUSIONS

There were no mortalities or clinical signs. Body weight, food intake, water consumption, ophthalmoscopy and haematology were unaffected by treatment. Glucose, total protein, albumin and creatinine were slightly and equivocally increased in high dose females. There was a reduction of urinary pH in high dose males. Gross pathology revealed no changes attributable to glyphosate treatment however animals at all dose levels showed increased incidence and severity of "cellular alteration" (deep basophilic staining and enlargement of cytoplasm) in the parotid salivary gland of both sexes at 1000 mg/kg bw/day and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes.

Assessment and conclusion by applicant:
In this study, the test item glyphosate was administered to Sprague-Dawley rats via the diet at dose levels of 0, 30, 300 or 1000 mg/kg bw/day for 13 weeks according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Dosing Sprague-Dawley rats via the diet for 13 weeks with up to and including 1000 mg/kg bw/day glyphosate produced increased incidence and severity of 'cellular alteration' (deep basophilic staining and enlargement of cytoplasm) in the parotid salivary gland of both sexes at 1000 mg/kg bw/day and at 300 mg/kg bw/day in males. Incidence only was increased at 300 mg/kg bw/day in females and 30 mg/kg bw/day in both sexes. In addition, there were slight equivocal increases in glucose, total protein, albumin and creatinine in females receiving 1000 mg/kg bw/day and reduced urinary pH in males receiving 1000 mg/kg bw/day.

A clear NOAEL could not be established in this study since the number of animals showing cellular alteration in the parotid salivary glands was markedly increased in all treated groups in both sexes following a dose-related pattern. However, this finding was not accompanied by a significant increase in salivary gland weight. Moderate and severe histological alteration was confined to the male and female rats of the high -dose groups. The other treatment related effects like clinical chemistry and urinalysis changes were also noted at the top dose level only. Therefore, it appears reasonable to establish the NOAEL in this study at 300 mg/kg bw/day.

Assessment and conclusion by RMS:
The RMS considers this study as acceptable as the deviations from the current version are due to the fact that the study was aligned to an older version of OECD TG 408. In contrast to the previous assessment and the proposal by the applicant, the NOAEL of 300 mg/kg bw/day is not agreed.

The RMS proposes a LOAEL of 30 mg/kg bw/day (the lowest dose tested), based on the following:

Histopathology revealed a statistically significant increased incidence of parotid cellular alterations in the salivary gland of both sexes at 1000 mg/kg bw/day. The finding was described by study author as deep basophilic staining and enlargement of cytoplasm. The incidence of this finding was 100% for males (compared to 30% in control) and 90% for females (compared to 20% in control). The severity grade of finding was minimal to severe in males, and minimal to moderate in females.

Also at 300 mg/kg bw/day a statistically significant increased incidence of parotid cellular alteration was observed in both sexes. The incidence was the same as for the high dose animals, but the severity grade was lower when compared to the high dose group. At 300 mg/kg bw/day the severity grade of finding in animals was very mild to mild (except for a single male animal which showed moderate cellular alteration).

At the lowest dose, 30 mg/kg bw/day, the increased incidence of parotid cellular alteration was statistically significant but only for females. The incidence was 70% in males (compared to 30% in control) and 80% in females (compared to 20% in control) and the severity grade of findings was minor (mostly very mild).

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.

In this study, no increase of salivary gland weights was reported. However, salivary gland weight was determined for the parotid, submaxillary and sublingual glands together. In this case, the conclusion that no effect was seen on salivary gland weight might be blurred as three different glands are weighted together whereas the effect only occurs in one of the glands (parotid mostly, submaxillary in some other studies).

As for this study no data is available on the parotid gland weight, the **RMS proposes to set the LOAEL at the lowest dose level of 30 mg/kg bw/day as a precautionary approach although the severity grade of findings observed at this dose level was minimal (very mild).**

Cellular alterations in the parotid gland were also reported in a NTP study in F344 rats (Chan and Mahler, 1992, TOX9551954). However, this study was not submitted. The applicant is requested to submit this study with an OECD summary and an evaluation of the results including the mechanistic study on the salivary gland.

In the previous assessment in the RAR (2015), the following was concluded by the RMS DE:
The study was not summarized in the RAR. However, the previous assessment from the DAR was considered acceptable. A NOAEL of 300 mg/kg bw/day was set based on decreased body weight gain in males, decreased urine pH, some changes in clinical chemistry parameters in females and cellular alterations in parotid salivary glands in both sexes.

B.6.3.2.7. Oral 90-day toxicity study in rats – study 7

Data point	CA 5.3.2/012
Report author	[redacted]
Report year	1990
Report title	Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat
Report No	[redacted]-900914
Document No	Not reported
Guidelines followed in study	OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	No sensory reactivity was examined; haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining cholesterol, HDL, LDL, blood urea nitrogen, T4, T3 and TSH; organ weights of the brain, epididymides, heart, ovaries, pituitary gland, prostate (seminal vesicles and coagulating glands), spleen, thyroid gland, thymus and the uterus were not determined; histopathology was performed without coagulating glands and vagina. No rationale for target dose selection

	is provided. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Not included in the previous assessments of glyphosate
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered unacceptable due to poor homogeneity of the test substance in the diet and uncertainties regarding the exact achieved dose levels in the study.

Executive Summary

Groups of male and female CD rats were dosed with glyphosate technical over a 90 to 92 day period. The test chemical was administered in the diet at levels of 0, 2000, 5000 and 7500 ppm (equivalent to 0, 129.1, 320.7 and 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 and 647.3 mg/kg bw/day for females). All concentrations of test compound included in the diet were readily consumed by the animals.

Animals were observed for mortality, clinical signs, body weight, food consumption, test substance intake, ophthalmoscopy, haematology, clinical chemistry, organ weights, gross necropsy and histopathology.

No deaths occurred in the control or any of the test groups throughout the study. Observations on the animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examination. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed background abnormalities only. Food consumption was decreased in males and females at the top dose. There was no adverse effect on organ weights or on organ weight/body weight ratios. Studies carried out on the terminal bleeds on the following haematology parameters - white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit, platelets, neutrophils, lymphocytes, monocytes, eosinophils and basophils - showed no compound-related adverse effects.

Measurement of the coagulation responses - prothrombin time and activated partial thromboplastin time - showed no compound-related adverse effect. Blood glucose was increased in males treated at 7500 ppm. Clinical chemistry analyses showed some group(s) differing significantly from the control for one or other parameter. Besides blood glucose, a progressive effect of dose on response was not seen for any parameter.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at the various dose levels could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

The decreased food consumption (of more than 10% compared with controls) in males and females and an increase in blood glucose in males at 7500 ppm was considered adverse by the RMS. However, as the study is not considered acceptable, no NOAEL is proposed by the RMS.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Yellowish (transparent)
Lot/Batch#:	0190 A
Purity:	98.1%
Stability of test compound:	Stable at room temperature in a dry vermin proof room

2. **Vehicle and/ or positive control:** Diet / none
3. **Test animals:**

Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Ca. 6 – 7 weeks
Sex:	Male and female
Weight at dosing:	♂ 233 – 310 g; ♀ 161 – 216 g
Acclimation period:	Approx. 3 – 4 weeks
Diet/Food:	Special quality control powdered diet (SDS Ltd, Witham, Essex, U.K.).
Water:	Bottled mains tap water, *ad libitum*
Housing:	Individually in flat bottomed polypropylene cages with stainless steel lids
Environmental conditions:	Temperature: 19 – 28 °C Humidity: 33 – 70% Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1990-03-21 to 1990-06-22

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 10 Sprague -Dawley CD rats per sex for a minimum of 90 days. Dietary target concentrations were 0, 2000, 5000 and 7500 ppm (equivalent to 0, 129.1, 320.7 or 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 or 647.3 mg/kg bw/day for females).

Table 6.3.2-1: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted] 1990): Study design

Test group	Dietary concentration [ppm]	Achieved dietary concentration [ppm]	Test substance intake [mg/kg bw/day]	Males	Females
Control	0		♂: 0; ♀: 0	10	10
Low	2000	1514	♂: 129.1; ♀: 174.3	10	10
Mid	5000	3422	♂: 320.7; ♀: 441.6	10	10
High	7500	5363	♂: 464.7; ♀: 647.3	10	10

Analysis of the test diet
Premixes of glyphosate in powdered diet were prepared in a stainless steel commercial food processor (Robot Coupe). Each pre-mix was diluted with untreated diet to give the correct final concentration. Mixing of the pre-mix with untreated diet was carried out by using a drum hoop mixer (Engelsmann, Germany). Samples from each batch of diet containing glyphosate were assayed in The National Food Centre laboratory by HPLC following suitable extraction procedures to establish concentration and homogeneity of the active ingredient.

Mortality
Animals were checked twice daily for mortality and moribundity.

Clinical observations
Each animal in each group was observed daily for toxic responses to the administered dose. In addition all cages were checked at the start and end of each day.

A full clinical examination was carried out on each animal at weekly intervals.

Body weight
For each animal, body weight was measured before dosing (Day 0), weekly thereafter and at terminal sacrifice.

Food consumption
Diet consumption was monitored at weekly intervals.

Ophthalmoscopy
The eyes of each animal in each group were examined for any defects before the study and were subjected to full ophthalmological examination at the end of the dose period (Day 83 for males and Day 84 for females). Animals were subjected to ophthalmoscopy in the order of initial random selection. 15 to 30 min before examination the eyes of each animal were treated with one drop of mydriatic (Trademark Mydriacyl, Alcon). Examination was carried out using an indirect ophthalmoscope (Fisons All-Purpose).

Haematology and clinical chemistry
Samples were taken by cardiac puncture under anaesthesia from animals of each group/sex prior to termination.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, prothrombin time, activated partial thromboplastin time, platelet count, total white blood cell count and differential white blood cell count.

Clinical chemistry
The following parameters were determined: Gamma glutamyl transpeptidase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine (Crea), total protein (TP), albumin (Alb), glucose (Glu), total bilirubin (T.Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and inorganic phosphorus (P).

Sacrifice and pathology
All animals were subjected to a full gross necropsy which included examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

Organ weights
The following organs were weighed: Adrenals, kidneys, liver and testes.

Histopathology
Histopathological examinations of the following organs and tissues were made on sections stained with haematoxylin and eosin for the control and high dose group: Adrenals, aorta, brain, epididymides, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), harderian glands, lacrimal glands, kidneys, liver, lungs, lymph node, mammary gland, muscle, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, midthoracic and lumbar), spleen, sternum, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, sternum with bone marrow, femur, and uterus.

Additionally, kidneys, liver and heart were examined from mid dose animals.

Statistics
Data was analysed using the General Linear Model ANOVA of SAS. Initial analysis for the effect of dose group on a given parameter was made by using ANOVA; this was followed by Duncan's Multiple Range Test. This test identifies dose-groups which are not statistically different. The level of significance for comparison between means was $p \leq 0.05$. As the above tests showed no differences or minor differences between groups, with no evidence of any dose-related effect, no further tests were carried out.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS

Each dose level of each batch of glyphosate/diet mixture was assayed following preparation to establish concentration and homogeneity of active substance. Each batch was sampled at weekly intervals for analysis of glyphosate content. Diet samples were analysed to assess the stability of glyphosate in the diet. Due to a delay in

assay development at the testing facility (The National Food Centre) the diet samples were not assayed at the required time. Final analysis revealed that the glyphosate/diet samples were stable for a period in excess of the feeding period.

Please refer to Table 6.3.2.7-1 for details on the achieved dosages which have been re-calculated based on food consumption and body weight data (see tables below).

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

No compound-related effects were seen in any animal for the dose groups throughout the test period.

One male rat in the 2000 ppm dose level received a cut on its right shoulder during the second week of the study. This was due to the rat scratching against the grid in the bottom of the cage. The grid was replaced and the cut had healed totally within nine days.

One male rat in the 7500 ppm dose group was noticed as somewhat unwell on study day 82. On study day 85 this animal was observed to have grossly overgrown incisors; the roof of the mouth was infected; the eyes appeared bloody and he had obvious signs of weight loss. The incisors were trimmed and improvement was obvious within 48 hours. Weight gain recommenced and the rat was eating normally at the termination of the study.

One male rat and one female rat escaped from their respective cages on study day 59. Both rats were recaptured and returned to their cages. All cages were secured and there were no further escapees. Body weight data confirmed that the female rat was pregnant. This rat was not removed from the study and littered on study day 80. Clinical chemistry, coagulation, body weight, haematology and organ weights were recorded, but the data from week 9 is not included in the reporting and statistics for this study.

D. BODY WEIGHT

While there are slight differences in group mean body weights the differences did not reach statistical significance for male or female animals at any time during the 90 day dosing period. There is no evidence that the compound had an adverse effect on growth for either sex.

Table 6.3.2-2: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted] 1990): Summary of mean body weights [g]

	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Week 0	268.20 ± 22.69	270.00 ± 18.73	269.70 ± 10.17	267.60 ± 17.37	198.40 ± 6.40	194.80 ± 14.21	191.30 ± 15.36	193.10 ± 11.20
Week 1	318.30 ± 25.79	323.00 ± 22.02	322.00 ± 14.69	318.30 ± 18.60	214.00 ± 7.59	209.50 ± 17.85	205.80 ± 13.85	204.60 ± 13.83
Week 2	364.30 ± 32.06	365.20 ± 20.49	368.90 ± 17.07	357.60 ± 23.58	236.20 ± 10.11	233.90 ± 25.99	228.80 ± 15.82	224.80 ± 16.23
Week 3	403.40 ± 37.67	405.20 ± 26.86	408.30 ± 20.49	393.40 ± 29.12	252.80 ± 10.40	251.40 ± 21.87	247.70 ± 19.25	240.80 ± 17.55
Week 4	438.90 ± 40.98	436.90 ± 28.39	443.80 ± 25.35	426.70 ± 37.97	266.70 ± 8.74	262.60 ± 21.22	258.70 ± 23.61	253.60 ± 19.35
Week 5	462.60 ± 42.23	458.60 ± 36.34	462.90 ± 27.65	449.00 ± 35.24	267.70 ± 10.93	270.40 ± 23.32	267.70 ± 25.41	263.70 ± 25.70
Week 6	491.30 ± 46.56	488.70 ± 36.48	493.30 ± 24.73	476.50 ± 40.89	287.90 ± 12.78	277.50 ± 21.56	271.90 ± 33.02	272.40 ± 23.20
Week 7	511.90 ± 52.04	497.90 ± 40.42	509.50 ± 26.76	487.60 ± 32.34	293.90 ± 14.83	286.80 ± 20.70	288.50 ± 32.30	278.50 ± 23.13
Week 8	533.00 ± 56.25	518.20 ± 42.85	524.60 ± 29.85	506.90 ± 41.70	299.70 ± 14.49	294.70 ± 24.59	290.30 ± 31.32	283.70 ± 24.24
Week 9	547.00 ± 56.01	525.80 ± 42.80	536.90 ± 29.37	516.90 ± 42.64	310.56 ± 12.84	302.20 ± 27.33	296.80 ± 31.57	288.90 ± 23.52

Table 6.3.2-2: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted] 1990): Summary of mean body weights [g]

	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Week 10	563.30 ± 65.58	549.10 ± 40.39	556.60 ± 29.86	537.20 ± 44.60	316.44 ± 17.77	308.50 ± 23.45	301.90 ± 30.98	294.80 ± 21.53
Week 11	583.90 ± 58.85	565.70 ± 40.46	572.10 ± 31.60	551.90 ± 47.16	326.11 ± 21.54	317.90 ± 24.79	309.70 ± 33.92	300.10 ± 21.53
Week 12	593.80 ± 65.84	572.70 ± 42.32	580.20 ± 32.91	555.80 ± 56.89	333.56 ± 19.33	320.60 ± 27.93	313.30 ± 38.63	305.10 ± 23.25
Day 90	612.30 ± 59.80	587.90 ± 46.26	597.20 ± 32.53	572.90 ± 55.67	339.00 ± 18.21	329.60 ± 27.96	324.50 ± 37.86	310.60 ± 24.65

E. FOOD CONSUMPTION

There is an indication of very slight decreased diet consumption for the high dose groups for both sexes.

Table 6.3.2-3: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted] 1990): Summary of food consumption data [g/rat/week]

	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Week 1*	201.99 ± 18.73	↑203.55 ± 21.14	↑203.88 ± 15.37	↑202.03 ± 13.87	245.51± 10.13	↓236.24 ± 17.94	↓239.99 ± 9.41	↓238.39 ± 13.59
Week 2	216.90 ± 21.67	↓215.07 ± 19.62	↑219.98 ± 14.57	↓206.07 ± 18.25	158.76± 11.35	↑162.20 ± 23.25	↓157.51 ± 14.41	↑162.49 ± 30.05
Week 3	220.91 ± 25.79	↑224.12 ± 26.02	↑222.68 ± 13.21	↓217.33 ± 22.00	160.57± 15.51	↑162.49 ± 20.20	↓157.60 ± 16.11	↓155.12 ± 10.56
Week 4	217.44 ± 26.71	↓215.43 ± 26.96	↑219.73 ± 19.12	↓212.70 ± 28.79	163.03± 15.24	↓162.39 ± 8.75	↓162.38 ± 16.01	↓154.75 ± 9.17
Week 5	208.44 ± 19.84	↑212.13 ± 23.79	↑214.24 ± 14.95	↑215.73 ± 24.32	148.61± 15.41	↓147.44 ± 11.36	↑151.05 ± 16.40	↓145.06 ± 11.28
Week 6	214.91 ± 22.26	↑218.33 ± 27.11	↑217.65 ± 17.34	↓213.03 ± 25.50	155.33± 16.37	↓150.97 ± 10.31	↓154.85 ± 15.28	↓148.03 ± 11.18
Week 7	215.76 ± 28.97	↓208.55 ± 27.26	↑216.24 ± 17.22	↓203.22 ± 17.42	152.70± 18.83	↓148.49 ± 15.39	↑154.71 ± 21.90	↓147.44 ± 12.92
Week 8	224.01 ± 26.42	↓216.23 ± 25.08	↓212.56 ± 17.17	↓209.93 ± 24.54	151.43± 15.77	↓149.53 ± 17.10	↓146.32 ± 14.25	↓137.81 ± 9.51
Week 9	215.13 ± 18.61	↓209.21 ± 33.61	↓208.44 ± 16.67	↓202.62 ± 21.44	156.08± 13.84	↑158.13 ± 13.39	↓150.56 ± 10.72	↓144.35 ± 9.70
Week 10	227.52 ± 25.15	↓225.76 ± 23.07	↓217.89 ± 17.69	↓216.18 ± 25.08	162.39± 18.70	↓157.39 ± 10.40	↓152.52 ± 11.91	↓144.64 ± 6.60
Week 11	228.34 ± 23.36	↓222.43 ± 23.61	↓224.36 ± 15.65	↓215.50 ± 30.29	172.89± 33.06	↓159.77 ± 12.79	↓160.95 ± 28.90	↓144.55 ± 6.20
Week 12	215.02 ± 20.61	↓213.30 ± 27.63	↓212.75 ± 17.33	↓193.32 ± 37.77	164.82± 24.12	↓150.27 ± 16.78	↓147.08 ± 17.79	↓135.45 ± 15.10
Day 90	179.17 ± 18.38	↓169.80 ± 22.47	↓176.10 ± 18.17	↓169.76 ± 20.22	126.74± 17.48	↓122.00 ± 11.12	↓123.36 ± 12.27	↓113.42 ± 7.52

* Week 1 female food intake figures as reported by the author appear out of expected range.

F. OPHTHALMOSCOPY

For most animals no abnormalities were recorded. The findings are those one would expect in this strain of rats at this age. There is no evidence of a compound related adverse effect.

Observations noted were:

Males: One control animal had vacuoles at 6 o'clock in the cornea of the left eye. One animal of the low dose group had vacuoles in the central region of the cornea of the right eye and on animal of the low dose group at 12 o'clock in the cornea of the left eye. One animal of the mid dose group showed corneal vacuoles at 3 o'clock in the right eye and one animal of the mid dose group had a pinhead posterior polar opacity in the lens of the right eye. One animal of the high dose group had bloody tears in the left eye (Adnexa) and one animal of the high dose group had nasal quadrant keratitis in the cornea of the right eye.

Females: Two animals of the control group showed increased luminescence in the right and left eye respectively (early retinal atrophy). One animal of the low dose group had a pinhead posterior opacity in the lens of both eyes, another animal of the low dose group showed increased luminescence in both eyes (early retinal atrophy) and one animal of the low dose group showed increased luminescence in the right eye (early retinal atrophy). One animal of the high dose group had conjunctivitis in the left eye.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Males: For the parameter RBC the Duncan test showed a statistically significant difference between the high dose group and the other three dose groups. The mean value for the high dose group was lower than the means for the other three dose groups. The mean value for all dose groups was within the normal range ($4.41 – 8.01 \times 10^6/mm^3$) for rats of this age and strain (Data supplied by [redacted]

For the parameter Haematocrit there was a statistically significant difference between the high dose group and the other three dosage groups. All dose groups including the control had a mean value slightly below the range (40.44 – 45.1 %) normally measured in male rats of this strain and age at [redacted]

For the parameter Monocytes the Duncan test shows a significant statistical difference between the low dose group and the other three dose groups. The control, intermediate and high dose groups were statistically similar. There was no evidence of a treatment-related effect.

Females: No statistical differences were seen for White Blood Cells (WBC), Haemoglobin (HGB), Platelets (PLT), Neutrophils (NP), Lymphocytes (LC), Eosinophils (EP) and Basophils (BP).
For the parameter Red Blood Cells (RBC) in the female animals the low dose group was significantly different from the control. The mean values for the intermediate and high dose group were similar to the control. All values were close to the normal range ($4.42 – 6.70 \times 10^6/mm^3$) for rats of that age and strain. (Data supplied by [redacted]). There was no progressive effect of treatment observed.

For the parameter Haematocrit the mean values for the low dose group was significantly different from the control. The mean values for the intermediate and high dose group were statistically similar to the control group using the Duncan test. All dose groups including the control group had a mean slightly below the range (37.5 – 47.3 %) normally measured in rats of this strain and age at [redacted]

For the parameter Monocytes the low dose group is significantly different from the control using the Duncan test. The intermediate and high dose levels are statistically similar to the control. ANOVA revealed no treatment related effect. There was no progressive effect of treatment observed.

All other haematological parameters were within the range of the control animals.

There was no statistical difference between the control and the dosage groups for either sex for the parameters Prothrombin time and Activated Partial Thromboplastin Time.

Table 6.3.2-4: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted] 1990): Selected haematological findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	**2000**	**5000**	**7500**	**0**	**2000**	**5000**	**7500**
Red blood cell count [x10^6/mm^3]	7.65 ± 0.49	↓7.54 ± 0.77	↓7.56 ± 0.49	**↓6.91* ± 0.57**	6.16 ± 0.67	**↑7.00** ± 0.63**	↑6.69 ± 0.42	↑6.32 ± 0.53

Table 6.3.2-4: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat (1990): Selected haematological findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Haematocrit [%]	38.85 ± 2.00	↓38.29 ± 3.76	↑39.10 ± 2.80	**↓35.63* ± 2.52**	33.11 ± 3.58	**↑37.47** ± 3.00**	↑35.78 ± 2.05	↑34.34 ± 2.59
Monocytes [%]	1.30 ± 0.82	**↓0.60* ± 0.84**	↑1.50 ± 0.85	↑2.00 ± 1.49	0.22 ± 0.44	**↑1.20** ± 0.42**	↑0.90 ± 1.20	↑0.80 ± 0.92

* Statistically significant compared to the other groups (ANOVA/Duncan test);
** Statistically significant compared to control (ANOVA/Duncan test)

Blood clinical chemistry
In the case of Ca levels in the male rats the mean value for the intermediate dose group (5000 ppm) was significantly different from the control. The mean value for the low dose group and the high dose group were statistically similar to the control value and there was no evidence of a dose-related effect.
For Na levels in the male rats ANOVA showed a significant effect of treatment. However, examination of the data shows an apparent suppression of the mean Na value for the control animals. The mean Na values for all groups seem somewhat low - only the value for the high dose group (7500 ppm) falls within the normal range (139 – 146 mmol/L) established for animals kept at

Statistical differences were also seen for mean Cl values. The mean value for the high dose group differs significantly from the mean value for the control and low dose groups. The mean chloride level for the control group was just below the lower end of the normal range; (94.5 – 112.0 mmol/L Data), whilst the values for all other groups were within the normal range.
For glucose concentrations ANOVA showed a significant effect of treatment. Reference to the Duncan test shows the control and low dose groups differing significantly form the medium and high dose groups. However, the values for all groups are within the range established for similar rats at

All other clinical chemistry parameters were in the range of the control group.

In the case of all parameters assayed, there was no statistical difference between the dosage groups and the controls at any dose level for the female rats.

In general there is no evidence of a toxic effect of glyphosate in male and female rats on the Clinical Chemistry parameters measured.

Table 6.3.2-5: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat 1990): Selected clinical chemistry findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Calcium [mmol/L]	2.34 ± 0.18	↑2.39 ± 0.16	**↑2.50** ± 0.17**	↑2.48 ± 0.09	2.69 ± 0.15	↓2.64 ± 0.18	↓2.61 ± 0.12	↓2.63 ± 0.19
Sodium [mmol/L]	128.20 ± 11.05	**↑128.70* ± 10.20**	**↑135.90* ± 10.16**	↑139.30 ± 6.41	139.63 ± 3.66	↓138.38 ± 6.55	↑141.13 ± 5.00	↑140.00 ± 3.56
Chloride [mmol/L]	93.67 ± 7.70	↑96.56 ± 6.93	↑98.60 ± 3.61	**↑103.00* ± 2.39**	99.00 ± 3.03	↑103.13 ± 8.87	↓100.20 ± 7.09	↓99.44 ± 4.59
Glucose [mmol/L]	7.66 ± 0.68	7.66 ± 0.68	**↑8.41§ ± 0.54**	**↑8.76§ ± 0.96**	8.79 ± 0.93	↓8.52 ± 0.63	↑8.69 ± 0.57	↑8.71 ± 0.84

* Statistically significant compared to control and low dose group (ANOVA/Duncan test);
** Statistically significant compared to control (ANOVA/Duncan test);
§ Statistically significant compared to control and high dose group (ANOVA/Duncan test)

H. NECROPSY

Organ weights/ body weight ratios
The organ weights (liver, kidneys, adrenals, and testes) in male animals showed no statistical differences between the dosage groups and the control group.
In the female animals the group mean value for the liver and the adrenals showed no statistical differences between the dosage groups and the control group. ANOVA showed that the mean weight of the kidneys for the control group was statistical different from the three dose groups. The mean values for the three dose groups were statistically similar. There was no progressive treatment-related effect observed.

Table 6.3.2-6: Glyphosate Technical: 90 Day Oral Toxicity Study in the Rat [redacted] 1990): Selected organ weight findings (week 13)

Parameter	Dose group [ppm]							
	Males				Females			
	0	2000	5000	7500	0	2000	5000	7500
Kidneys	4.00 ± 0.42	4.26 ± 0.49	4.36 ± 0.44	3.97 ± 0.48	**2.56* ± 0.19**	2.23 ± 0.25	2.27 ± 0.14	2.35 ± 0.15

* Statistically significant compared to the dose groups (ANOVA)

For male animals the statistics showed no treatment-related effect on body weight at death or on the organ weight/body weight ratios for liver, adrenals and testes. An effect of treatment was observed for kidneys with the mean weight for the low dose group differing significantly from the control. The Duncan test showed that for male animals the 2000 ppm dose group was significantly different from the control but the 5000 ppm and 7500 ppm were similar to the control group. There was no indication of a dose-related effect in the kidney weight/body weight ratios.

For the kidney weight/body weight ratio in the female animals a similar pattern was seen. The Duncan test reveals that the control group was statistically different from the low dose group; however, the control group, intermediate group and the high dose group were statistically similar. No dose-related effect of treatment was observed. For female animals there were no statistical differences between the dose groups for the liver weight/body weight ratio and the adrenal weight/body weight ratio or for body weight at death.

Gross pathology
The few macroscopic abnormalities observed at necropsy are common in rats and were not considered related to administration of the test material.

Histopathology
The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at the various dose levels could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

III. CONCLUSIONS

No deaths occurred in the control or any of the test groups throughout the study. Observations on the animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examination. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed background abnormalities only. There was no adverse effect on organ weights or on organ weight/body weight ratios. Studies carried out on the terminal bleeds on the following haematology parameters - white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit, platelets, neutrophils, lymphocytes, monocytes, eosinophils and basophils - showed no compound-related adverse effects.

Measurement of the coagulation responses - prothrombin time and activated partial thromboplastin time - showed no compound-related adverse effect.

Clinical chemistry analyses showed some group(s) differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound-related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at the various dose levels could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

Glyphosate technical appeared to be without adverse effect when fed in the diet to CD rats over a 90 – 92 day period. The no adverse effect level was found to be in excess of 7500 ppm in the diet.

Assessment and conclusion by applicant:
In this study, groups of male and female CD rats were dosed with glyphosate technical over a 90 to 92 day period. The test material was administered in the diet at levels of 0, 2000, 5000 or 7500 ppm (equivalent to 0, 129.1, 320.7 or 482.1 mg/kg bw/day for males and 0, 174.3, 441.6 or 647.3 mg/kg bw/day for females) according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).
Glyphosate technical appears to be without adverse effect when fed in the diet to CD rats over a 90 – 92 days period. The no observed adverse effect level was found to be in excess of 7500 ppm in the diet (equivalent to 482.1 mg/kg bw/day for males and 647.3 mg/kg bw/day for females).

Assessment and conclusion by RMS:
The RMS considers this study as unacceptable due to poor homogeneity of some batches and uncertainties regarding the achieved dose levels in the study. Based on the dietary analysis the achieved concentrations seem to be much lower than the target concentrations, however, these were measured 8-16 weeks after administration. Therefore, it is unclear which dose level was achieved during the study.

In contrast to the notifier, a decreased food consumption (of more than 10% compared with controls) in males and females and an increase in blood glucose in males at 7500 ppm (equivalent to 482.1 mg/kg bw/day for males and 647.3 mg/kg bw/day for females) was considered adverse by the RMS.

As the study is not considered acceptable, no NOAEL is proposed by the RMS.

This study has not been considered in the previous assessments of glyphosate.

B.6.3.2.8. Oral 90-day toxicity study in rats – study 8

Data point	CA 5.3.2/013
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: 90 Day Oral Toxicity study in the Rat
Report No	[redacted]-891002
Document No	Not reported
Guidelines followed in study	OECD 408 (1981); EEC Directive 87/302 EEC (Page 8)
Deviations from current test guideline (OECD 408, 2018)	Haematology was performed without determining reticulocyte count; clinical chemistry was performed without determining cholesterol, HDL, LDL, T4, T3 and TSH. Organ weight of the brain, epididymides, heart, ovaries, prostate with seminal vesicles, spleen, thyroid, thymus, pituitary gland and uterus was not determined; histopathology was performed without bone/bone marrow, coagulating glands, gross lesions, lymph nodes, male mammary glands, seminal vesicles and vagina. No rationale for target dose selection is provided (highest dose lower than recommended 1000 mg/kg bw/day). Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability / Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable with restrictions based on the deviations mentioned above.

Executive Summary
Groups of individually housed male and female rats were dosed with glyphosate technical over a 90 – 92 day period. The chemical was administered in the diet at levels of 0, 2000, 3000, 5000, or 7500 ppm (equivalent to 0, 100, 150, 250 or 375 mg/kg bw/day for males and females). All concentrations of test compound included in the diet were readily consumed by the animals.

Mortality, clinical observations, ophthalmology, body weight and food consumption were recorded during the treatment period. In the final week of dosing ophthalmological examination were made and blood taken for determination of haematological and blood chemistry parameters. At necropsy, organ weights were determined and histopathological examinations were performed on processed tissues.

No deaths occurred during the study. Observations on the live animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examinations. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed no abnormalities. There was no adverse effect on organ weights or on organ weight \ body weight ratios. Studies carried out on the terminal bleeds showed no adverse effects on the following haematology parameters: white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit and platelets. Investigation of the differential leucocyte count showed some groups differing significantly from the control. However, a progressive effect of dose on response was not seen for any parameter. The occasional minor differences seen are considered not to be compound related.

Measurement of the coagulation responses showed no compound related adverse effect. Clinical chemistry analyses showed some group mean values differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at up to 7500 ppm could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Grey solid
Lot/Batch #:	L1656
Purity:	97.1%
Stability of test compound:	Stable for the duration of the study

2. Vehicle and/ or positive control: Diet / none

3. Test animals:

Species: Rat

Strain:	CD
Source:	[redacted]
Age:	Ca. 6 – 7 weeks
Sex:	Male and female
Weight at dosing:	♂ 119 – 242 g; ♀ 116 – 172 g
Acclimation period:	14 days
Diet/Food:	Standard powdered diet
Water:	Bottled mains tap water, *ad libitum*
Housing:	Individually in flat bottomed polypropylene cages with stainless steel lids
Environmental conditions:	Temperature: 18 – 23 °C Humidity: 40 – 70% Air changes: Not reported 12 hours light / dark cycle

B: Study design and methods

In life dates: 1989-04-12 to 1989-10-02

Animal assignment and treatment
The test material was offered on a continuous basis in the basal diet to groups of 10 CD rats per sex for 90 – 92 days. Dietary concentrations were 0, 2000, 3000, 5000, 7500 and 7500 (satellite group) ppm. The satellite groups were fed untreated for further 5 weeks.

Table 6.3.2-1: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat [redacted] 1989): Study design

Test group	Dietary concentration [ppm]	Number of animals	
		Males	Females
Control	0	20	20
Control (satellite)	0	10	10
Low	2000	10	10
Intermediate low	3000	10	10
Intermediate high	5000	10	10
High	7500	10	10
High (satellite)	7500	10	10

Analysis of the test diet
Samples from each batch of diet containing glyphosate were assayed in the Sponsor's laboratory by HPLC following suitable extraction procedures.

Mortality
Viability was checked once per day.

Clinical observations
All animals were observed daily for signs of toxicity. In addition all cages were checked at the start and end of each day. A full clinical examination was carried out on each animal at weekly intervals.

Body weight
For each animal body weight was measured before dosing (Day 0), weekly thereafter and at terminal sacrifice.

Food consumption and test substance intake
For the first 21 days of the study food consumption was monitored at 3 day intervals. For the remainder of the study diet consumption was monitored at weekly intervals.

The body weight values of the first three weeks of treatment were only given in 3-Day intervals. Therefore it was not possible to calculate the achieved test substance intake in mg/kg bw/day from these values. For the conversion from ppm to mg/kg bw/day the factor of 20 for elder rats set by Derelanko, M.J. (2014, Handbook of Toxicology) was used to access a worst case scenario.

Ophthalmoscopy examination
The eyes of each animal in each group were subjected to full ophthalmological examination during (Day 58 – males, Day 57 – females) and at the end of the dose period (Day 89 – males, Day 88 – females). 15 – 30 min before examination the eyes of each animal were treated with one drop of mydriatic. Examination was carried out using an indirect ophthalmoscope.

Haematology and clinical chemistry
All animals in the dosage groups were bled in random sequence by cardiac puncture under anaesthesia for haematology, coagulation and clinical chemistry analysis.

Another group of animals of the same age range was selected before the start of the study and bled to establish normal ranges for haematology, coagulation and clinical chemistry parameters.

Haematology:
The following parameters were determined: White blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), platelets (PLT) and differential white blood cell count. Additionally, the plasma from the citrate blood sample was assayed for Prothrombin time manually (Quick's method) and for Activated Partial Thromboplastin Time - also by a manual method.

Clinical chemistry
The following parameters were determined: Calcium, phosphorus, chloride, sodium, potassium, fasting glucose, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), gamma glutamyl transpeptidase (gGT), urea, albumin, blood creatinine, total serum protein and total bilirubin.

Sacrifice and pathology
All animals were subjected to a full gross necropsy which included examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents.

Organ weights
The following organs were weighed: Adrenals, kidneys, liver and testes.

Histopathology
The following tissues were processed and examined histopathologically from 12 male and 13 female Control animals and all High dose animals. In addition, examinations of liver, kidneys and lungs were performed on all groups.

The following tissues were investigated: Adrenals, aorta, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), Harderian glands, lacrimal glands, kidneys, liver, lungs, mammary gland (female), muscle, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, peripheral and sciatic nerve, seminal vesicles, skin, spinal cord (cervical, midthoracic and lumbar), spleen, stomach, salivary glands, testes (with epididymides), thymus, thyroid, trachea, urinary bladder, sternum with bone marrow, femur, and uterus.

The satellite groups were observed for further 5 weeks after treatment. Delayed effects were not seen during the additional 5 week observation period. At the end of this period animals were subjected to the following termination procedures: Haematology, coagulation, gross necropsy and organ weight measurements.

Statistics
Data was analysed using the General Linear Model <ANOVA> and NPAR1WAY <WILCOXON> procedures of SAS. Initial analysis for the effect of dose group on a given parameter was made by using ANOVA; this was followed by Duncan's Multiple Range Test. This test identifies dose groups which are not statistically different.

In addition data for gamma glutamyl transpeptidase, total bilirubin, monocytes, eosinophils and basophils were analysed using the Wilcoxon Rank Sum Test. Where a dose group related effect was seen further Wilcoxon tests were carried out to identify groups differing significantly from the control. The level of significance for comparison

between means was $p \leq 0.05$. As the above test showed no differences or minor differences between groups, with no evidence of any dose related effect, the further tests outlined in the protocol were not carried out.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES

The achieved test substance concentrations are summarised in the following table:

Table 6.3.2-2: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat [redacted] 1989): Achieved test substance intake

Test group	Dietary concentration [ppm]	Achieved test substance intake [mg/kg bw/day]*	
		Males	Females
Control	0	0	
Control (satellite)	0	0	
Low	2000	100	
Intermediate low	3000	150	
Intermediate high	5000	250	
High	7500	375	
High (satellite)	7500	375	

* Recalculation of dose levels by applying the appropriate diet conversion factor of 20 as published by Derelanko (The Toxicologist's Pocket Handbook, 2nd Ed., 2008)

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

No compound related effects were seen in any animal for the dose groups throughout the test period.

Three animals did show injuries of a mechanical nature. One female rat in the 3000 ppm dose group showed a cut on the head. This cut was food-hopper related and the animal showed immediate signs of improvement once the hopper was changed, and the cut had healed completely within eleven days.

One male rat in the 7500 ppm dose group developed a red bald patch on its back. The reason for this was not obvious. The animal made good body weight gains and showed no other adverse effects. Fur had regrown completely by four weeks.

One female rat in the 7500 ppm satellite dose group was reported to have slight hair loss on the head. The bald patch was 0.5 cm^2, approximately and remained for ca. 11 days when a regain in fur growth was noted. No other obvious abnormalities were seen in this animal.

D. BODY WEIGHT

Throughout the bulk of the feeding period the dosage groups did not differ significantly from the control group. There is no evidence that the compound had an adverse effect on growth for either sex.

E. FOOD CONSUMPTION

There is no evidence of decreased diet consumption for any dose group for either sex.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no statistical differences between the control and the dosage groups for male and female rats for the parameters WBC, RBC, HGB, HCT and PLT.

In the case of the differential leucocyte count for the male animals no treatment related effect was seen for eosinophils, basophils and neutrophils. For monocytes both ANOVA and the Wilcoxon test showed the 2000 ppm group to be significantly different from all other groups. However, there was no dose-related effect as the control, the 3000, 5000 and 7500 ppm dose groups were not statistically different.
For the parameter lymphocytes the 2000 and 5000 ppm dose groups were significantly elevated with respect to the control. However, the 3000 and 7500 ppm groups were not significantly different and a progressive effect of dose was not seen.

The parameters monocytes, eosinophils and basophils showed no treatment-related effect either on the ANOVA or Wilcoxon tests, for the female animals. For the parameter neutrophils the 3000 ppm group showed a significant decrease with respect to the control. The other dose groups were not different from the control and there was no evidence of a progressive effect.

Table 6.3.2-3: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat [redacted] 1989): Selected haematological findings (means ± SD)

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
Monocytes [%]	4.25 ± 1.02	**2.9* ± 0.99**	4.10 ± 1.37	4.20 ± 1.03	4.00 ± 0.94	3.32 ± 0.95	3.80 ± 1.48	3.90 ± 1.10	2.90 ± 0.99	3.90 ± 1.20
Lymphocytes [%]	78.60 ± 3.12	**83.00* ± 4.24**	81.30 ± 3.16	**83.80* ± 3.16**	80.10 ± 3.67	78.68 ± 3.32	74.20 ± 6.94	82.60 ± 4.99	82.10 ± 6.51	80.90 ± 4.95
Neutrophils [%]	15.30 ± 3.08	12.70 ± 3.47	13.10 ± 3.85	10.90 ± 2.13	13.40 ± 3.69	16.84 ± 3.48	19.60 ± 6.82	**11.80* ± 3.58**	13.30 ± 5.72	13.70 ± 3.71

* Significant effect of treatment

There were no statistical differences between the control and the dosage groups for either sex for the parameter Prothrombin time.
There were no statistical differences between the control and the dosage groups for female rats for the parameter Activated Partial Thromboplastin Time (APTT). However, in the case of male animals, the 7500 ppm dose group was statistically different from the 2000, 3000 and 5000 ppm dose groups. The mean value for this parameter for CD rats kept at [redacted] is 22.25 seconds with a standard deviation of 3.45. Both the control and 7500 ppm groups in this experiment were significantly different from the mean baseline value. The reason for this anomaly is not clear.

Table 6.3.2-4: Glyphosate Technical: 90 Day Oral Toxicity study in the Rat ([redacted] 1989): Selected clinical chemistry findings (means ± SD)

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
APTT [s]	27.3 ± 5.5	**↓20.2* ± 2.5**	**↓20.5* ± 2.5**	**↓23.3* ± 2.6**	↑27.7 ± 5.6	23.5 ± 9.4	↓20.7 ± 2.0	↓20.4 ± 2.9	↓22.7 ± 3.1	↓20.3 ± 1.6

* Significant effect of treatment

Blood clinical chemistry
In the case of the following parameters - gGT, calcium, total protein, potassium and urea - there was no statistical difference between the dosage groups and the controls at any dose level (Statistical analysis was not performed on gGT values for male rats, due to small sample numbers).

For the parameter ALT all male dose groups were slightly but significantly elevated with respect to the control. However there was no progressive increase with dose level (the 2000, 3000 and 7500 ppm groups were not significantly different from each other). For all dose groups the values seen were within the normal range (39 – 183 u/L) for male CD rats.

For female rats the ALT levels for the 3000 ppm and the 5000 ppm group did not differ significantly from the control group. The 2000 ppm and 7500 ppm animals did not differ significantly from each other but were significantly elevated with respect to the control group. There was no progressive increase with dose level. For all dose groups the values seen were within the range (30 – 262 u/L) for female CD rats of the same age group.

For the parameter AST, there was no significant difference between any of the dose groups and the control for the male animals. For the female animals the 2000 ppm group was significantly elevated. There was no significant difference between the mean levels of all other groups and the mean level for the control. There was no evidence of a progressive increase with dose.

For total bilirubin, the dose groups in the case of the male animals had significantly lower mean levels than the control group. The reason for this result is not clear. For the female animals there was no significant difference between any dose group and the control.

For the parameter total protein, the group mean values did not differ from the control for either sex.

For the parameter albumin, the 5000 ppm dose group had a slightly but significantly lower mean value than the control. The other groups – 2000, 3000 and 7500 ppm - were not statistically different from the control. The albumin levels for all male animals including the control rats were considerably lower than the values for the female animals. For the female rats no dose group had a mean value significantly different from the control group mean value.

For the parameter sodium, the 5000 ppm male group mean value was slightly but significantly lower than the control group level. The levels for the other groups were not statistically different from the control value. There was no evidence of a progressive effect with dose. The group mean values for the female rats were not significantly different from the control value.

In the case of creatinine values the dose groups were not statistically different for female animals. For male animals the values for the 2000, 3000 and 5000 ppm dose groups are significantly lower than the control and 7500 ppm values. There is no evidence of a dose-related effect.

Table 6.3.2-5: 90 Day Oral Toxicity study in the Rat [redacted] 1989): Selected clinical chemistry findings

Parameter	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
ALT [u/L]	64.35 ± 8.63	↑**87.56* ± 19.22**	↑**80.56* ± 12.94**	↑79.11 ± 6.47	↑**89.70* ± 11.21**	65.80 ± 11.01	↑**100.90 * ± 45.26**	↑76.29 ± 9.23	↑76.67 ± 9.25	↑**94.33* ± 25.18**
AST [u/L]	150.55 ± 24.65	↑166.44 ± 36.95	↑179.33 ± 32.34	↑150.67 ± 24.15	↓149.40 ± 30.32	123.07 ± 21.62	↑**181.90 * ± 87.72**	↑**145.43 * ± 25.83**	↑133.89 ± 25.98	↑126.67 ± 26.41
Albumin [g/L]	31.65 ± 2.21	↓31.22± 1.64	↑31.67 ± 1.12	↓**29.00* ± 1.87**	↑31.80 ± 1.75	42.27 ± 3.47	↓39.44 ± 2.60	↑42.29 ± 3.15	↓41.89 ± 3.95	↓40.33 ± 3.87
Total bilirubin [µM/L]	4.45 ± 0.51	↓**1.67*± 0.50**	↓**1.89* ± 0.33**	↓**1.89* ± 0.60**	↑**3.60* ± 1.26**	4.36 ± 0.50	↓4.22 ± 0.83	↑4.43 ± 0.79	↓4.33 ± 0.50	↓4.11 ± 0.60
Sodium [mM/L]	144.25 ± 8.88	↓142.89 ± 2.32	↓142.33 ± 2.45	↓**137.25 * ± 7.80**	↓143.40 ± 5.56	141.54 ± 3.48	↑144.88 ± 3.48	↑145.71 ± 2.14	↑145.67 ± 2.24	↑141.57 ± 11.01
Creatinine [µM/L]	45.40 ± 3.16	↓**39.89* ± 2.26**	↓**38.63* ± 2.56**	↓**39.88* ± 3.23**	↑45.78 ± 5.52	41.00 ± 4.76	↑44.43 ± 5.91	↓39.67 ± 3.61	↑42.67 ± 3.20	↑45.33 ± 4.08

* Significant effect of treatment

H. NECROPSY

Organ weights

For liver, kidneys, adrenals and testes in male animals, there were no statistical differences between the dosage groups and the control.
In female animals, there were no statistical differences between the dosage groups and the control for kidneys and adrenals. In the 2000 ppm dose group, the mean liver weight is statistically different from the control, but is well within the normal range (Mean ± 2 × SD) for control animals. The 3000, 5000 and 7500 ppm dose groups were not statistically different from the control group.

Table 6.3.2-6: 90 Day Oral Toxicity study in the Rat [redacted] 1989): Selected organ weights

Organ	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
Liver [g]	18.24 ± 2.96	↓18.04± 3.67	↑18.79± 3.43	↓17.51 ± 3.12	↑19.23± 3.40	10.26 ± 1.51	↑**11.91*** **± 1.28**	↑10.35± 1.29	↑10.79 ± 1.46	↑10.40± 1.12

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

For male animals the statistics showed no treatment related effect on body weight at death or on the organ/body weight ratios examined (liver, kidney, adrenals and testes).

For female animals ANOVA showed no treatment related (i.e. test compound-related) effect on body weight at death or on the organ/body weight ratios.

At the 2000 ppm dose the p-value was 0.064. The Duncan test showed this group to be significantly different from the control. However, there was no progressive effect of treatment. All of the higher dose levels did not significantly change the liver/body weight ratio.

Table 6.3.2-7: 90 Day Oral Toxicity study in the Rat [redacted] 1989): Selected organ/body weight ratios

Organ	Dose group [ppm]									
	Males					Females				
	0	2000	3000	5000	7500	0	2000	3000	5000	7500
Liver/body weight ratio	0.04258 ± 0.0037	↓0.03994 ± 0.0067	↑0.04335 ± 0.0045	↓0.04185 ± 0.0040	↑0.04297 ± 0.0066	0.04174 ± 0.0054	↑0.04659 ± 0.0043	↑0.04218 ± 0.0328	↑0.04360 ± 0.0039	↑0.04191 ± 0.0033

Gross pathology
In the 2000 ppm dosage group one male rat was reported to have enlarged intestines. The epididymal fat pad appeared red and irritated and an abscess-like growth was found attached to the caecum.

In the 3000 ppm dosage group, one female rat had a distended uterus. A second female in this group had an enlarged uterine wall.

No obvious abnormalities were seen in the other animals for the dosage groups.

Histopathology
The range of histopathological findings in the study animals was within the expected spectrum of background pathology. The dosed animals were not distinguishable from the control animals. There were no histopathological findings suggestive of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.

Satellite group
Satellite animals were fed normal powdered diet for 5 weeks following the end of the live phase of the main study. There was no evidence of a delayed effect during this period. No adverse clinical effects were noted.

No compound related abnormalities were seen at gross necropsy. For the haematology and coagulation parameters the satellite control and 7500 ppm dose groups were statistically similar. The body weight, organ weight and organ weight/body weight ratios were also statistically similar for the two groups. No further investigations were carried out.

III. CONCLUSIONS

No deaths occurred during the study. Observations on the live animals showed no compound-related or dose-related adverse effects - either at the weekly clinical examinations or the ophthalmological examinations. There was no compound related adverse effect on growth. Gross necropsy at the end of the dosing period showed no abnormalities. There was no adverse effect on organ weights or on organ weight \ body weight ratios. Studies carried out on the terminal bleeds showed no adverse effects on the following haematology parameters: white blood cell counts, red blood cell counts, haemoglobin concentration, haematocrit and platelets. Investigation of the differential leucocyte count showed some groups differing significantly from the control. However, a progressive effect of dose on response was not seen for any parameter. The occasional minor differences seen are considered not to be compound related.

Measurement of the coagulation responses showed no compound related adverse effect. Clinical chemistry analyses showed some group mean values differing significantly from the control for one or other parameter. However, a progressive effect of dose on response was not seen for any parameter. Overall no compound related adverse effect was seen.

The range of histopathology findings in the study animals was such as would be expected within a normal group of rats of this age range. The animals receiving glyphosate at up to 7500 ppm could not be distinguished on the basis of the histopathology findings. There was no evidence of specific target organ cytotoxicity attributable to administration of the test substance at any dose level.
The no adverse effect level was greater than 7500 ppm in the diet.

3. Assessment and conclusion

Assessment and conclusion by applicant:
In this study, groups of male and female CD rats were administered glyphosate technical via the diet at dose levels of 0, 2000, 3000, 5000, 7500 or 7500 (satellite group) ppm (equivalent to 100, 150, 250 or 375 mg/kg bw/day for males and females) over a period of 90 – 92 days according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

There were no treatment-related adverse effects on survival, clinical signs, body weight, haematology, clinical chemistry and histopathology. Therefore, the no observed adverse effect level (NOAEL) was in excess of 7500 ppm in the diet (equivalent to 375 mg/kg bw/day in males and females) under the conditions of this study.

Assessment and conclusion by RMS:
The RMS considers this study as acceptable but with restrictions (reliable with restrictions) considering the mentioned deviations (refer to first table).
The RMS does agree with the NOAEL of ≥ 7500 ppm as proposed by the notifier (equivalent to 375 mg/kg bw/day in males and females) as no adverse and dose-related effects were observed in this study. This conclusion is also in agreement with the previous evaluations (DAR and RAR (2015)).

B.6.3.2.9. Oral 90-day toxicity study in rats – study 9

Data point	CA 5.3.2/014
Report author	[redacted]
Report year	1987
Report title	90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats

Report No	[redacted] 7375
Document No	Not reported
Guidelines followed in study	Not reported, but in general compliance to OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Clinical signs were not recorded daily; no sensory reactivity was examined; haematology was performed without determining prothrombin time; clinical chemistry was performed without determining HDL, LDL, T4, T3 and TSH; organ weights of the adrenals, brain, heart, ovaries, pituitary gland, prostate (seminal vesicles and coagulating glands), spleen, thyroid gland, thymus and the uterus were not determined; histopathology was performed without coagulating glands and vagina. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary
Glyphosate (Lot XLG 161) was administered to Sprague-Dawley rats at target levels of 0, 1000, 5000 or 20000 ppm in the feed for approximately three months. Analyses to verify the stability of the test material both neat and when mixed with the diet, the diet homogeneity, and concentrations of the test material in the diet were perfomed with satisfactory results. Overall averages of dietary concentrations for the study were 950, 4600 or 19000 ppm for the low, middle and high levels, respectively. Overall averages for consumption of test material at the low, middle and high levels, respectively, were 63, 317 or 1267 mg/kg bw/day for males and 84, 404 or 1623 mg/kg bw/day for females.

The group size was 12 animals per sex and dose group. The animals were examined for mortality (daily), clinical signs, body weight, food consumption (weekly), ophthalmoscopy (before treatment and termination), and haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology at termination.

No evidence of toxicological effects was observed in any parameter examined.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate (T860067)
Description:	White powdery solid
Lot/Batch#:	Lot XLG 161
Purity:	95.21 %
Stability of test compound:	At the start of the study, the analysed purity of the test substance was 95.4 ± 2.9% and three months after the end of the study at 93.1 ± 2.1%.

2. Vehicle and/or positive control: Diet / none

3. Test animals:

Species:	Albino rat
Strain:	Sprague-Dawley
Source:	[redacted]

Age:	ca. 6 weeks
Sex:	Male and female
Weight at dosing:	♂ 206 g; ♀ 141 g
Acclimation period:	16 days
Diet/Food:	Ralston Purina RODENT CHOW No. 5002
Water:	Water (sodium zeolite-conditioned St. Louis public water supply), *ad libitum*
Housing:	Individual suspended stainless steel cages, over paper bedding
Environmental conditions:	Temperature: 21 – 23 °C Humidity: 35 – 60% Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1986-11-20 to 1987-02-26

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 12 Sprague-Dawley rats per sex for a minimum of 90 days. Dietary target concentrations were 0, 1000, 5000 or 20000 ppm. Analysis of the test diets revealed an average dose of 0, 950, 4600 or 19000 ppm which is equivalent to 0, 63, 317 or 1267 mg/kg bw/day for males and 0, 84, 404 or 1623 mg/kg bw/day for females.

Table 6.3.2-1: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats [redacted] 1987): Study design

Test group	Dietary target concentration [ppm]	Average dietary concentration [ppm]	Average achieved dose[mg/kg bw/day]	Males	Females
Control	0	0	♂: 0; ♀: 0	12	12
Low	1000	950	♂: 63; ♀: 84	12	12
Mid	5000	4600	♂: 317; ♀: 404	12	12
High	20000	19000	♂: 1267; ♀: 1623	12	12

Analysis of the test diet
Test Material Stability: Determined prior to and after the in-life portion of the study.

Homogeneity of Diet Mixtures: Analysis of duplicate samples from top, middle, and bottom of mixer for lowest and highest levels (determined once during the study).

Diet Mixture Stability: Analysis of samples kept refrigerated (closed container, 33 days) or at ambient temperature (open container, 6 and 14 days)

Dietary Level Verification: Extraction of diets with water/chloroform; analysis by liquid chromatography with UV/VIS detector; all dietary levels for first 6 weeks, one level/week thereafter

Mortality
Animals were checked twice daily for mortality and moribundity.

Clinical observations
Detailed observations for clinical signs of toxicity were performed weekly.

Body weight
The weight of each animal was recorded once weekly.

Food consumption
The quantity of food consumed by each animal was recorded once each week.

Ophthalmoscopy
Ophthalmoscopic examination was performed by indirect ophthalmoscopy on all animals before treatment and near termination on all surviving animals.

Haematology and clinical chemistry
Samples were taken from the posterior *vena cava* of all anesthetised animals from each group/sex at termination. Food and water was withheld for approximately 18 hours prior to blood collection.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total white blood cell count, differential white blood cell count (thin blood smears on labelled glass slides prepared, stained with Wright's stain, and examined microscopically) and reticulocyte count (a portion of the EDTA-treated sample mixed with a vital stain (methylene blue), a slide prepared and examined microscopically).

Clinical chemistry
The following parameters were determined: Alkaline phosphatase (ALP), aspartate amino transferase (AST), Alanine aminotransferase (ALT), creatinine (Crea), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin, glucose (Glu), total cholesterol (Chol), total bilirubin (T.Bi), direct bilirubin, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P).

Urinalysis
Urine samples were collected from all animals via metabolism trays for approximately eighteen hours. Food and water was withheld during urine collection. The following parameters were measured: Specific gravity, glucose, ketones, blood, pH, protein, urine sediment, bilirubin and urobilinogen.

Sacrifice and pathology
All animals were killed and necropsied. External and internal investigations were performed on opened internal cavities and organs *in situ* and then removed. Hollow organs were opened and examined.

Organ weights
The following organs were weighed: Kidneys, liver and testes (with epididymides).

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals. The following tissues were investigated: lesions and masses, adrenals, aorta, bone with bone marrow, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), harderian gland, kidneys, liver, lungs, mammary gland, mesenteric lymph node, muscle, nasal turbinates, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, sciatic nerve, seminal vesicles, skin, spinal cord (cervical, thorax, lumbar), spleen, stomach, submaxillary salivary gland, submandibular lymph node, testes (with epididymides), thymus, thyroid/parathyroid, trachea, urinary bladder and uterus (with cervix).
Additionally, kidneys, liver and lungs were examined from low and mid dose animals.

Statistics
The following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls:

Dunnett's Multiple Comparison Test (two-tailed): body weights, food consumption, non-categorical clinical pathology data, absolute organ weights.

Mann-Whitney Test with Bonferroni Inequality Procedure: Organ weight/ body weight ratios.

Fisher's Exact Test with Bonferroni Inequality Procedure: Incidence of microscopic lesions.

Other statistical routines used for some data were: Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population and Grubb's Test to detect outliers.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS

Results of analyses for test material stability conducted over a span of time exceeding the length of the study indicated the neat test material was stable. The homogeneity of the diet mixtures was determined to be adequate. The coefficients of variation were 3.0% at 1000 ppm dose level and 6.7% at the 20000 ppm dose level. The stability of the test material/diet mixtures was demonstrated for the low and high levels, stored in open containers at room temperature for up to 14 days, and stored in closed containers in a refrigerator for 33 days (with glyphosate levels ranging from 90-112% of the target dose). Weekly analyses of the test material in the diet were performed on all levels for the first 6 weeks and on one level each week thereafter. Please refer to the table above for the average achieved dietary concentration and for the calculated achieved dose levels in mg/kg bw/day.

B. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

C. CLINICAL OBSERVATIONS

There were no clinical signs in the control and treated groups that were considered to be due to administration of glyphosate.

D. BODY WEIGHT

There were no notable intergroup differences in either sex.

E. FOOD CONSUMPTION

There were no notable intergroup differences in total food consumed in either sex at any time.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no deviations in haematology values which were attributed to administration of the test material. Statistically significant increases in lymphocytes in low and mid-level males and in WBC count in mid-level males were not a part of a dose-related trend, were within the normal range for rats of this age, and, therefore, were attributed to biological variation.

Table 6.3.2-2: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats [redacted] 1987): Selected haematological findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	950	4600	19000	0	950	4600	19000
Differential white blood cell count – Lymphocytes [10^3/mm^3]	7.49 ± 2.19	↑**9.92* ± 2.34**	↑**11.03** ± 2.04**	↓6.58 ± 1.87	4.35 ± 0.98	↑4.42 ± 1.62	↑4.86 ± 1.71	↓3.78 ± 0.68
White blood cell count [10^3/mm^3]	10.6 ± 2.1	↑12.8 ± 2.3	↑**14.0** ± 2.7**	↓10.3 ± 3.6	5.7 ± 1.2	5.7 ± 1.8	↑6.4 ± 2.0	↓5.5 ± 1.6

* Statistically significant compared to control (Dunnett's test; $p \leq 0.05$);
** Statistically significant compared to control (Dunnett's test; $p \leq 0.01$)

Blood clinical chemistry

Serum inorganic phosphate and potassium were elevated in all treatment groups, and glucose was mildly elevated in mid and high level males when compared to control animals. These elevations did not increase in a dose -related manner and were either within normal ranges (potassium - all levels both sexes, inorganic phosphate - all female groups, glucose - all male groups) or close to the upper limits of normal (inorganic phosphate – male groups). Therefore, these elevations were not considered toxicologically relevant. Elevations in BUN and alkaline phosphatase in high level males were attributed to elevations in one rat from this group and not attributed to the test material. Terminal investigations for this particular rat (M3 007) revealed multiple findings in the kidney, ureter and bladder (bladder distension, numerous calculi in kidney, bladder and ureter, pyelonephritis in the kidney) indicative of urolithiasis and associated bacterial infection. This was considered to be an isolated case unrelated to administration of glyphosate.

Table 6.3.2-3: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats [redacted] 1987): Selected clinical chemistry findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	950	4600	19000	0	950	4600	19000
Calcium [mg/dL]	11.3 ± 0.26	↑11.7 ± 0.24	↑**12.0** ± 0.22**	↑11.4 ± 0.83	11.1 ± 0.27	↑11.3 ± 0.54	11.1 ± 0.31	↓11.0 ± 0.29
Glucose [mg/dL]	175 ± 23.6	↑184 ± 20.4	↑**248** ± 26.4**	↑**205** ± 36.6*	123 ± 20.4	↓120 ± 24.3	↑140 ± 17.3	↓115 ± 14.0
Phosphate [mg/dL]	8.4 ± 0.56	↑**9.4** ± 0.86*	↑9.2 ± 0.61	↑**9.4** ± 1.3*	7.2 ± 0.84	↑**9.2** ± 0.99**	↑**9.1** ± 0.87**	↑**8.4** ± 0.97**
Sodium [mEq/L]	161 ± 2.6	151 ± 1.6	↓149 ± 1.6	↓**146** ± 9.1*	148 ± 1.4	↑147 ± 1.0	146 ± 1.3	↑147 ± 2.9
Potassium [mEq/L]	6.9 ± 0.79	↑7.6 ± 1.1	↑**8.2** ± 1.2*	↑**8.0** ± 1.4*	7.4 ± 0.73	↑**8.4** ± 1.4*	↑8.4 ± 1.6	↑8.1 ± 0.98
Blood urea nitrogen [mg/dL]	17.2 ± 6.6	↓16.4 ± 2.0	↓14.6 ± 1.6	↑31.3 ± 53.7§	20.1 ± 2.6	↓18.1 ± 2.1	↓16.9 ± 1.9	↑20.4 ± 6.2
Alkaline phosphatase [IU/L]	204 ± 39.6	↑207 ± 27.7	↓199 ± 36.6	↑284 ± 166§	137 ± 32.0	↓112 ± 43.9	↓108 ± 23.1	↑148 ± 33.7

* Statistically significant compared to control (Dunnett's test; $p \leq 0.05$);
** Statistically significant compared to control (Dunnett's test; $p \leq 0.01$);
§ Animal M3 007 was not excluded for the statistical comparisons.

H. URINALYSIS

There were no abnormalities in urine measurements attributed to the test material. Statistically significant changes in specific gravity and pH of mid-level males were not considered toxicologically relevant.

Table 6.3.2-4: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats [redacted] 1987): Selected urinalysis findings

Parameter	Dose group [ppm]							
	Males				Females			
	0	950	4600	19000	0	950	4600	19000
pH	6.1 ± 0.5	↑6.2 ± 0.4	↑**6.8** ± 0.3*	↑6.4 ± 0.9	5.8 ± 0.4	↑5.7 ± 0.4	↑5.7 ± 0.5	↓5.5 ± 0.5
Specific gravity	1.058 ± 0.015	↑1.060 ± 0.010	↓**1.040** ± 0.012*	↓1.041 ± 0.020	1.063 ± 0.019	↑1.068 ± 0.024	↑1.068 ± 0.025	↑1.068 ± 0.015

* Statistically significant compared to control (Dunnett's test; $p \leq 0.05$)

I. NECROPSY

Organ weights

There were no statistically or toxicologically significant differences in organ weights.

Gross pathology

The few macroscopic abnormalities observed at necropsy are common in rats and were not considered related to administration of the test material.

Histopathology
Microscopically, three high level male rats had chronic or active inflammation of the pancreatic islets which extended into the acinar parenchyma in two animals. This lesion is relatively common in this strain of rat and was not considered treatment-related in this study. The incidence of all other microscopic lesions observed in treatment groups was not significantly different from that of their respective controls.

Table 6.3.2-5: 90 Day Study of Glyphosate Administered in Feed to Sprague/Dawley Rats [redacted] 1987): Selected histopathological findings

Parameter		Dose group [ppm]							
		Males				Females			
		0	950	4600	19000	0	950	4600	19000
Pancreas	Acinar atrophy/ degeneration	1	-	-	3	-	-	-	-
	Fibrosis, interstitial, islets	0	-	-	3	-	-	-	-
	Inflammation, islets	0	-	-	3	-	-	-	-
	Inflammation, acinar	0	-	-	2	-	-	-	-
	Mononuclear infiltrate	2	-	-	2	1	-	-	2

III. CONCLUSIONS

Glyphosate (Lot XLG 161) was administered to Sprague-Dawley rats at target levels of 0, 1000, 5000 or 20000 ppm in the feed for approximately three months.

The animals were examined for mortality (daily), clinical signs, body weight, food consumption (weekly), ophthalmoscopy (before treatment and termination), and haematology, clinical chemistry, urinalysis, gross pathology, organ weights and histopathology at termination.

No evidence of toxicological effects was observed in any parameter examined. Therefore, a No Observable Effect Level (NOEL) for glyphosate, as administered in this study, was apparently greater than 20000 ppm.

Assessment and conclusion by applicant:
In this study, glyphosate was administered to Sprague-Dawley rats at target levels of 0, 1000, 5000 or 20000 ppm in the feed (equivalent to actual doses of approx. 0, 63, 317 or 1267 mg/kg bw/day for males and 84, 404 or 1623 mg/kg bw/day for females) for approximately three months in general compliance to OECD 408 (1981) and GLP (no certificate of the competent authority was provided).
No evidence of toxicological effects was observed in any parameter examined. Therefore, the NOAEL for glyphosate, as administered in this study, is ≥ 19000 ppm (actual dose; equivalent to 1267 mg/kg bw/day for males and 1623 mg/kg bw/day for females).

Assessment and conclusion by RMS:
The RMS does agree with the NOAEL of ≥ 19000 ppm as proposed by the notifier (actual dose; equivalent to 1267 mg/kg bw/day for males and 1623 mg/kg bw/day for females) as no adverse and dose-related effects were observed in this study.
This conclusion is also in agreement with the previous evaluations (DAR and RAR (2015)).

B.6.3.2.10. Oral 90-day toxicity study in rats – study 10

Data point:	CA 5.3.2/015
Report author	[redacted]
Report year	1985

Report title	Subacute Oral Toxicity (90 Days) to Rats of Glyphosate (Technical) of [redacted]
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not reported, similar to OECD 408 (1981)
GLP	Not reported
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Groups of ten Wistar rats per sex and dose were administered glyphosate (purity not reported) at dose levels of 0 (control group receiving the vehicle, i.e. 0.1 % Tween 80 in water), 300, 1200 or 2400 mg/kg bw/day for 90 days by oral gavage. In addition, a second group receiving the mid dose of 1200 mg/kg bw/day was sacrificed after a 30-day recovery period (reversal group). Animals were observed daily for signs of toxicity. Body weight and food consumption were determined regularly. Blood samples for haematological (red and white cell parameters) and clinical chemistry (total serum protein, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen and glucose) investigations were taken prior to treatment, on day 45 and on days 91 and 121 just prior to sacrifice. Urinalysis was also performed. All animals were subjected to gross pathological examination and extended histopathology (salivary glands, however, not examined). Organ weights were determined.
Short description of results:	There were no deaths during the study and no signs of toxicity were observed. Laboratory investigations and pathological examinations did not reveal indications of adverse effects. The only findings which could be attributed to treatment were a somewhat lower body weight gain and a reduced food intake becoming more apparent towards the end of treatment period in high dose males and females. Thus, the NOAEL in this 90–day gavage study was 1200 mg/kg bw/day.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supportive in the Monograph (2000) due to serious reporting deficiencies, e.g. the year when the study was performed, was not indicated in the original report. Furthermore, there was no information on the guideline followed and on GLP status available from the original report. Statistical analysis of the results was not reported. RAR (2015): The study was considered unacceptable due to serious reporting deficiencies, e.g. absence of statistical analysis. Report identification and dates of experimental work not given. Purity and Batch number of the test substance not reported. Therefore and since the study report is not available to GRG, this study is considered invalid by GRG. **Conclusion GRG:** The study is considered unacceptable, category 4b. **Conclusion AGG**: The study report has been made available to AGG by BVL. The RMS has evaluated the study and agrees with the previous conclusion that the study is not considered acceptable due to serious reporting deficiencies, e.g. absence of statistical analysis, report identification and dates of experimental work not given, and purity and batch number of the test substance not reported. The RMS agrees with the results reported above. At the top dose, an adverse and treatment-related decrease (>10%) in both mean body weight and food consumption compared with controls was seen in both males and females at termination. There were no treatment-related effects at the mid and low dose. However, no NOAEL is proposed as the study is not considered acceptable.

Reasons why the study report is not available for submission	The notifier has no access to this study report. The former RMS (BVL) has made the study report available to the current RMS.

B.6.3.2.11. Oral 90-day toxicity study in rats – study 11

Data point:	CA 5.3.2/016
Report author	Anonymous
Report year	1981
Report title	Glyphosate subchronic toxicological study
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not reported, similar to OECD 408 (1981)
GLP	No, pre-GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Groups of 10 Wistar rats per sex and dose were administered glyphosate (purity: 96.8%; manufacturer: [redacted]) for one or for three months, respectively, at dietary levels of 0, 1000, 3000 or 10000 ppm. Observations performed were: Mortality, clinical signs, body weight, food consumption, haematology, clinical chemistry, organ weight and histopathology.
Short description of results:	There was no mortality in this study and no clinical signs of toxicity. Body weight, body weight gain and food consumption were similar throughout the study groups. Haematology revealed a number of changes of which a reduced red blood cell count, an increase in leucocyte count at 3000 and 10000 ppm in both sexes and a higher platelet count in all treated male groups and in high and mid dose females were probably treatment-related. In females, blood glucose levels were increased at the highest dose level. Alkaline phosphatase activity was increased in both sexes at 10000 ppm and so were alanine aminotransferase and aspartate aminotransferase activities. At 10000 ppm, the liver weights were increased in both sexes and for the males round liver edges were reported. The lowest dietary level of 1000 ppm is considered the NOAEL in this study. For male and female rats receiving the test substance for three months, a mean daily compound intake of 102.0 or 105.4 mg/kg bw/day, respectively, was calculated for this dose group. At the mid dose of 3000 ppm, the mean daily compound intake was calculated at 284.0 and 376.8 mg/kg bw/day in males and females, respectively. At 10000 ppm, these were 1103.7 mg/kg bw/day and 1310.8 mg/kg bw/day, respectively.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supplementary in the Monograph (2000) due to serious reporting deficiencies. When the study was performed, GLP was not compulsory. Measurement of mean daily intake of test substance for all dose levels was not performed. The study was considered unacceptable in the RAR (2015). Therefore and since the study report is not available to GRG, this study is not considered to be reliable by GRG. **Conclusion GRG:** The study is considered unacceptable, category 4b. **Conclusion AGG**: The study report has been made available to AGG by BVL. The RMS has evaluated the study and agrees with the previous conclusion that the study is not considered acceptable as measurement of the test substance in the diet was not performed and therefore no information is available on the actual concentration of the test substance in the diet,

	and the homogeneity and the stability of the test substance. The study design is comparable to OECD 408 (1981). The RMS agrees with the results reported above and with the conclusion that there were no treatment-related effects at the lowest dietary level of 1000 ppm. However, no NOAEL is proposed as the study is not considered acceptable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. The former RMS (BVL) has made the study report available to the current RMS.

B.6.3.2.12. Oral 13-week toxicity study in mice – study 1

Data point	CA 5.3.2/017
Report author	[redacted]
Report year	1995
Report title	HR-001: 13-week Subchronic Oral Toxicity Study in Mice
Report No	[redacted] 94-0136
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Reticulocytes not counted, clotting not evaluated, total cholesterol but not HDL and LDL measured, urea not measured, no blood hormones (T3, T4 and TSH) measured; organ weights limited to brain, liver, kidneys, testes, adrenals and caecum; vaginal smears not taken; sensory reactivity to different stimuli was not evaluated. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408. Further, it should be noted that the highest dose tested (~6000-7000 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary

In order to evaluate the sub-chronic toxicity of HR-001 in mice, the test substance was administered by incorporating it into a basal diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females) for a period of 13 weeks.

50000 ppm group: Males showed a depressed body weight gain associated with lowered food consumption and food efficiency throughout the treatment period. Decreased food efficiency was also observed in females. In haematological examinations, females showed decreases in haematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC). Blood chemical examinations revealed increases of alkaline phosphatase (ALP) in males and females and inorganic phosphorous (P) in females. At necropsy, males and females revealed increased incidences of distention of the caecum. In organ weight analysis, males and females showed increases of absolute and relative weights of the caecum. Histopathologically, males showed an increase in incidence of cystitis of the urinary bladder.

10000 ppm group: Distention of the caecum was observed in one female at necropsy. In organ weight analysis, increasing tendencies were noted in absolute and relative weights of the caecum.

5000 ppm group: There were no treatment-related changes in either sex in any parameters.

The NOAEL of 3000 ppm (equivalent to 1221 mg/kg bw/day for males and 1486 mg/kg bw/day for females) is supported and according to the RMS this is based on reduced food consumption in the first week in males, increased alkaline phosphatase in both sexes, increased blood phosphorus in females, increased creatinine phosphokinase in females, distension of the caecum and increased absolute and relative caecum weight in both sexes, and an increased incidence of cystitis in the urinary bladder in males observed at the LOAEL of 50000 ppm (equivalent to 6295 mg/kg bw/day for males and 7435 mg/kg bw/day for females). The decreased body weight in males at the top dose was not considered adverse as the decrease was less than 10% compared with controls. In addition, a shift towards lower urinary pH was observed in all dose groups (significant in males, not significant in females), however, this effect was not considered adverse as this is due to acidic properties of the test substance and is therefore not considered a toxic effect. The effect on caecum distension and caecum weight observed at the mid dose is not considered adverse as these were not accompanied by histopathological changes.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	HR-001
Description:	White crystal
Lot/Batch#:	T-941209
Purity:	97.56%
Stability of test compound:	26/12/1994

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Mouse
Strain:	Crj:CD-1
Source:	[redacted]
Age:	5 weeks
Sex:	Male and female
Weight at dosing:	♂ 27.3 – 32.7 g; ♀ 22.4 – 25.8 g
Acclimation period:	9 days
Diet/Food:	MF Mash (Oriental Yeast Co., Ltd.)
Water:	Filtered and sterilized tap water, *ad libitum*
Housing:	3/cage, sexes separately in stainless steel cages 21.5 × 33.0 × 18.0 cm
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: 1995-01-10 to 1995-04-27

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 24 SPF ICR mice (Crj: CD-1) (12 males + 12 females) for a minimum of 90 days. Dietary concentrations were 0, 5000, 10000 or 50000 ppm

(equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females).

Table 6.3.2-1: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Study design

Test group	Dietary concentration [ppm]	Test substance intake [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	12	12
Low	5000	♂: 600.2; ♀: 765.0	12	12
Mid	10000	♂: 1221; ♀: 1486	12	12
High	50000	♂: 6295; ♀: 7435	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about 50 g each) of each does level taken from top, middle and bottom portions of the mixer at the first diet preparation. The control diet was also sampled (50 g each) and analysed to confirm that there was no contamination with the test substance. Concentrations of the test substance in test diets at all dose levels were monitored on the same amount of samples (50 g each) every 3 weeks during the study.

Mortality
Each animal was checked for mortality or signs of morbidity at least once daily during the treatment period.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. Group mean body weight was calculated for each dose group at each measurement. Final body weights were recorded for all animals before necropsy.

Food consumption and utilisation
Food consumption for each cage was measured weekly for a period of 3 consecutive days. Mean daily food consumption per animal in each cage was calculated by dividing the weekly food consumption by the number of animals per cage and by the number of days for measurement. Group mean food consumption (g/rat/day) was calculated at each measurement from the mean daily food consumption per animal in each cage.

Group mean chemical intake (mg/kg bw/day) was calculated from nominal dietary concentrations of the test substance, food consumption and body weight.

Group mean food efficiency for each dose group was calculated weekly from the ratio of the group body weight gain to group mean food consumption and expressed as percentage. Overall group mean efficiency throughout the treatment period was also calculated for all dose groups.

Ophthalmoscopic examination
Ophthalmological examinations including observation with a halogen ophthalmoscope were performed on all animals during acclimatisation period and on all surviving animals in the control and the highest dose groups from the main group at week 13. The following parameters were determined: Eyeball, cornea, anterior chamber, pupil and iris.

Haematology and clinical chemistry
After 13 weeks of treatment, all surviving animals were subjected to haematological examinations. The animals were laparotomised under anaesthesia following overnight fasting, and blood samples were withdrawn from the posterior vena cava using heparinised syringes. A part of each sample was poured into a cup treated with EDTA and subjected to the examinations.

The following parameters were determined with a fully automated haematology analyser: Haematocrit (Ht), haemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), total leukocyte count (WBC) and differential leukocyte count.

After 13 weeks of treatment, all surviving animals were subjected to blood biochemical examinations. Plasma samples obtained from the heparinised blood were used for examinations.

The following parameters were determined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca) and inorganic phosphorus (P).

Urinalysis
At 13 weeks of treatment, all surviving animals were subjected to urinalysis. Fresh urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was determined with a handy refractometer. Glucose, ketones, occult blood, pH, protein, and urobilinogen were semi-quantitatively analysed by Uro-labstix. Then animals were housed individually in metabolic cages overnight, and urine samples collected were examined for volume and appearance. Urinary sediments were also examined microscopically on these samples.

Sacrifice and pathology
Clinical pathology evaluations were also conducted. Selected organs were weighed at the scheduled necropsy (brain, liver with gall bladder, kidneys, adrenals, testes, caecum (including contents). Histopathological examinations were performed on selected tissues from all animals.
The following parameters were determined: Brain (cerebrum, cerebellum, pons and medulla), spinal cord (cervical, thoracic and lumbar region), sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow (sternum and femur), tibio-femoral joint, lymph nodes (cervical and mesenteric), heart, aorta, pharynx, salivary glands (submaxillary and sublingual), oesophagus, stomach (forestomach and glandular stomach), liver with gall bladder, pancreas, duodenum, jejunum, ileum, caecum, colon, rectum, head (including nasal cavity, paranasal sinuses, tongue, oral cavity and middle ears), larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, seminal vesicles, epididymides, coagulating glands, ovaries, uterus (including cervix), vagina, harderian glands, eyes, skeletal muscle, skin, mammary gland and all gross lesions.

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, food consumption, urine specific gravity, haematological parameters, blood biochemical parameters, and organ weights were evaluated by Bartlett's test for equality of variance. 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 (equal animal numbers among groups) or Scheffe's (unequal animal numbers among groups) 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 (equal animal numbers among groups) or Scheffe's type mean rank test (unequal animal numbers among groups) was applied. The data of urinalysis except for specific gravity were assessed by Mann-Whitney's U test. Fisher's exact probability test was used to analyse the data of clinical sign, mortality, ophthalmological examinations and incidences of gross lesions at necropsy and histopathological lesions.

II. RESULTS AND DISCUSSION

DIETARY ANALYSIS
Homogeneity of the test substance in diet was analysed on the samples taken from the top, middle, and bottom portions of the mixer at the first preparation. The coefficient variance for each test diet was 2.2% or less. The results indicated that a good homogeneity was obtained by the present preparation method. To confirm concentration of the test substance in the test diet, analyses were conduct ed at regular intervals. Mean concentration of the test substance in the test diets at nominal levels of 5000, 10000, and 50000 ppm were 4879, 9958, and 49632 (mean) ppm, respectively. Since the overall mean values were within 98-100% of the target concentrations, it was verified that the concentration of the test substance were within acceptable limits.

A. MORTALITY

There were no animals found dead or killed *in extremis* in any group during the treatment period.

B. CLINICAL OBSERVATIONS

There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.

C. BODY WEIGHT

In the 50000 ppm group, mean body weights of males were lower than those of the control from week 2 to the end of the treatment period. Mean body weight of males at week 13 was 91% of that of control. Body weights of females were comparable to the control during the treatment period.

In the groups treated at 10000 ppm or less, body weights of males and females were comparable to the controls during the treatment period.

Table 6.3.2-2: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Group mean body weights (selected weeks) and standard deviations

Dietary concentration [ppm]	Body weight [g] at week					
	0	2	6	8	12	13
Males						
0	30.1 ± 1.6	35.0 ± 2.2	39.1 ± 2.9	40.5 ± 2.9	43.0 ± 2.8	43.2 ± 3.1
5000	30.1 ± 1.6	↓34.6 ± 2.3	↑39.5 ± 2.7	↑41.1 ± 3.0	↑44.1 ± 3.3	↑44.2 ± 3.3
10000	30.1 ± 1.5	↑35.2 ± 2.1	↑39.5 ± 2.9	↑41.1 ± 3.4	↑43.8 ± 3.8	↑44.4 ± 3.8
50000	↓30.0 ± 1.6	**↓32.6* ± 2.0**	↓37.3 ± 1.8	↓37.9 ± 2.2	**↓39.1* ± 3.4**	**↓39.5* ± 3.3**
Females						
0	23.9 ± 0.9	26.6 ± 1.6	30.7 ± 2.7	32.1 ± 2.2	35.2 ± 2.7	34.8 ± 2.2
5000	23.9 ± 1.0	↑26.9 ± 1.7	↑31.0 ± 2.0	32.1 ± 2.4	↓34.7 ± 3.3	↓34.5 ± 3.6
10000	23.9 ± 1.0	↑27.1 ± 1.7	↑31.5 ± 3.9	↑33.8 ± 4.4	↑36.7 ± 5.6	↑37.0 ± 5.8
50000	23.9 ± 1.0	↓26.1 ± 1.6	↓30.0 ± 1.9	↓31.3 ± 2.2	↓33.0 ± 2.1	↓33.4 ± 2.2

* Significantly different from control group ($p < 0.05$)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

In males of the 50000 ppm group, a significant depression of food consumption was recorded at week 1 (28% decreased compared with the control group). Average food consumption of males during the treatment period was 94 % of the control value. Food consumption of females was comparable to the control. In the groups treated at 10000 and 5000 ppm, food consumption of males and females was comparable to that of the controls.

The average daily test substance intake during the treatment are shown in the following table:

Table 6.3.2-3: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Average test substance intake

Dose level [ppm]	Average test substance intake [mg/kg bw/day]	
	Males	Females
5000	600.2	765.0
10000	1221	1486
50000	6295	7435

In the 50000 ppm group, food efficiency of males and females was lower than that of the control animals at almost all measuring points during the treatment. Average food efficiency of males and females remained at 79% and 88% of the respective control value.

In the groups treated at 10000 and 5000 ppm, food efficiency in the treated groups of both sexes was comparable to that in the controls though some significant fluctuations were recorded sporadically.

Table 6.3.2-4: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted] 1995): Average food efficiency during the treatment period [body weight gain/food consumption × 100]

Dose level [ppm]	Average food efficiency [%]	
	Males	Females
0	2.9	2.6
5000	↑3.3	↓2.5
10000	↑3.2	↑3.1
50000	↓2.3	↓2.3

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological abnormalities in the animals of both sexes in the highest dose group and the control group.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

The significant changes (mean ± SD) observed after 13 weeks in the treated groups are summarised in the table below:

Table 6.3.2-5: HR-001: 13-week Subchronic Oral Toxicity Study in Mice ([redacted] 1995): Selected haematological findings

Parameter	Sex		Dose group [ppm]		
		0	5000	10000	50000
Haematocrit (Ht) [%]	female	43.6 ± 1.4	↓42.8 ± 2.4	↓42.5 ± 2.2	**↓40.0** ± 2.2**
Haemoglobin concentration (Hb) [g/dL]	female	14.1 ± 0.4	↓13.9 ± 0.7	↓13.8 ± 0.9	**↓13.0** ± 0.6**
Erythrocyte count (RBC) [10^6/mm^3]	female	8.71 ± 0.38	↓8.69 ± 0.57	↓8.57 ± 0.42	**↓8.05** ± 0.52**

** Significantly different from the control ($p < 0.01$, estimated by Dunnett's multiple comparison test)

In the high dose group, females showed significant decreases in haematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC), while males showed no significant differences from the control in any parameters. There were no significant differences in any parameters between the treated groups of 10000 ppm or less and the control of either sex.

Blood clinical chemistry

The significant changes (mean ± SD) observed in the treated groups are summarised in the following table:

Table 6.3.2-6: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Selected blood clinical chemistry findings

Parameter	Sex	Dose group [ppm]			
		0	5000	10000	50000
Alkaline phosphatase (ALP) [U/L]	Male	37 ± 8	37 ± 6	↑47 ± 9	**↑68** ± 16 (+84%)**
	Female	60 ± 15	↑69 ± 19	↑61 ± 11	**↑90** ± 29 (+50%)**
Glutamic pyrvic transaminase (GPT) [U/L]	Female	26 ± 7	↓22 ± 7	↓24 ± 7	**↓18** ± 3**
Creatine phosphokinase (CPK) [U/L]	Female	49 ± 14	**↑177** ± 239**	↑84 ± 102	**↑462** ± 894 (~9.4 times higher)**
Blood urea nitrogen (BUN) [mg/dL]	Female	23.4 ± 3.3	↑23.8 ± 3.2	**↑27.9** ± 3.6**	↑25.4 ± 3.6
Inorganic phosphorus (P) [mg/dL]	Female	5.4 ± 0.7	↓5.3 ± 0.7	↑5.8 ± 0.8	**↑6.9** ± 0.9 (+28%)**

** Significantly different from control group ($p < 0.01$)

In the 50000 ppm group, males and females showed a small but statistically significant increase in alkaline phosphatase (ALP). In females, creatine phosphokinase (CPK) and inorganic phosphorus (P) were significantly increased, while a significant decrease in glutamic pyrvic transaminase (GPT) was noted.

In the 10000 ppm group, females exhibited a small but statistically significant increase in blood urea nitrogen (BUN). There were no significant changes in any parameters in males.

In the 5000 ppm group, females showed a significant increase in CPK, while there were no significant changes in any parameters in males.

G. URINALYSIS

In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

Table 6.3.2-7: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Intergroup comparison of urinary pH (number of animals at pH value)

pH	Dose Group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000
5.0	-	-	-	3	-	-	-	-
6.0	-	2	4	8	7	5	5	12
6.5	1	5	3	1**	2	4	5	-
7.0	3	3	3	-	2	2	2	-
7.5	2	2**	2**	-	-	1	-	-
8.0	6	-	-	-	1	-	-	-

** Significantly different from the control group ($p < 0.01$)

H. NECROPSY

Organ weights

In the 50000 ppm group, males and females showed significant increases in both absolute and relative weights of the caecum. The absolute weights of the caecum of males and females were 238 % and 187 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 263 % or 195 % in males or females.

In the 10000 ppm group, absolute and relative weights of the caecum showed increasing tendencies in males and females. The absolute weight of the caecum of males and females were 115% and 122% of that of the respective control. For relative weight, the ratio of the value to the respective control was 111% or 117% in males or females.

In the 5000 ppm, there were no significant changes in any organ weights of males and females.

Table 6.3.2-8: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Intergroup comparison of caecum weight – absolute and relative to body weight

Organ		Dose group [ppm]							
		Males				Females			
		0	5000	10000	50000	0	5000	10000	50000
Caecum	Absolute [mg]	624 ± 86	↓609 ± 116	↑718 ± 177	**↑1484** ± 359 (+138 %)**	497 ± 96	↓474 ± 115	↑604 ± 123	**↑928** ± 163 (+87%)**
	Relative [%]	1.45 ± 0.19	↓1.38 ± 0.26	↑1.61 ± 0.33	**↑3.82** ± 1.15 (+163 %)**	1.43 ± 0.26	↓1.37 ± 0.30	↑1.67 ± 0.42	**↑2.79** ± 0.53 (+95%)**

Table 6.3.2-8: HR-001: 13-week Subchronic Oral Toxicity Study in Mice [redacted] 1995): Intergroup comparison of caecum weight – absolute and relative to body weight

Organ	Dose group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000

** Statistically significant from controls ($p < 0.01$)

Gross pathology
In the 50000 ppm group, males and females showed a significant increase in incidence of distention of the caecum (12/12 in males and 10/12 in females; 0/12 in males and females of the control group).

In the 10000 ppm group, distention of the caecum was observed in one female. There were no significant changes in incidence of any macroscopic lesions in males.

In the 5000 ppm group, there were no treatment-related abnormalities in males and females.

Histopathology
In the 50000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12; 0/12 of the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distention of the caecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the caecum.
In the 10000 and 5000 ppm groups, there were no significant differences in incidence of histopathological lesions from the control in either sex.

III. CONCLUSIONS

In order to evaluate the sub-chronic toxicity of HR-001 in mice, the test substance was administered by incorporating it into a basal diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm for a period of 13 weeks.

50000 ppm group: Males showed a depressed body weight gain associated with lowered food consumption and food efficiency throughout the treatment period. Decreased food efficiency was also observed in females. In haematological examinations, females showed decreases in haematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC). Blood chemical examinations revealed increases of alkaline phosphatase (ALP) in males and females and inorganic phosphorous (P) in females. At necropsy, males and females revealed increased incidences of distention of the caecum. In organ weight analysis, males and females showed increases of absolute and relative weights of the caecum. Histopathologically, males showed an increase in incidence of cystitis of the urinary bladder.

10000 ppm group: Distention of the caecum was observed in one female at necropsy. In organ weight analysis, increasing tendencies were noted in absolute and relative weights of the caecum.

5000 ppm group: There were no treatment-related changes in either sex in any parameters.

Based on these results, the no observable effect level, minimal toxic level and sure toxic level of HR-001 in ICR (Crj:CD-1) mice under the conditions of the present study were determined as follow.

	Males	Females
No observable effect level	5000 ppm (600.2 mg/kg bw/day)	5000 ppm (765.0 mg/kg bw/day)
Minimal toxic level	10000 ppm (1221 mg/kg bw/day)	10000 ppm (1486 mg/kg bw/day)
Sure toxic level	50000 ppm (6295 mg/kg bw/day)	50000 ppm (7435 mg/kg bw/day)

Assessment and conclusion by applicant:
In this study, glyphosate technical was administered to groups of male and female SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 600.2, 1221 or 6295 mg/kg bw/day for males and 0, 765.0, 1486 or 7435 mg/kg bw/day for females) for a period of 13 weeks according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Under the experimental conditions of the study, the NOAEL is considered to be 10000 ppm (equivalent to 1221 and 1486 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:
The RMS considers this study as acceptable as the deviations from the current version are due to the fact that the study was aligned to an older version of OECD TG 408. It should be noted that the study director was in fact [redacted], as in a 90-day rat study from the same laboratory. [redacted] performed the histopathological examinations and was apparently the report writer.

The main conclusions drawn are supported by the RMS, which is also in agreement with the conclusions of the previous assessment of glyphosate (see below). The NOAEL of 10000 ppm (equivalent to 1221 mg/kg bw/day for males and 1486 mg/kg bw/day for females) is supported and according to the RMS this is based on reduced food consumption in the first week in males, increased alkaline phosphatase in both sexes, increased blood phosphorus in females, increased creatinine phosphokinase in females, distension of the caecum and increased absolute and relative caecum weight in both sexes, and an increased incidence of cystitis in the urinary bladder in males observed at the LOAEL of 50000 ppm (equivalent to 6295 mg/kg bw/day for males and 7435 mg/kg bw/day for females). The decreased body weight in males at the top dose was not considered adverse as the decrease was less than 10% compared with controls. In addition, a shift towards lower urinary pH was observed in all dose groups (significant in males, not significant in females), however, this effect was not considered adverse as this is due to acidic properties of the test substance and is therefore not considered a toxic effect. The slight effect on caecum distension and caecum weight observed at the mid dose is not considered adverse as these were not accompanied by histopathological changes.

In the previous assessment in the RAR (2015), the following was concluded by the RMS DE:
"The study is considered acceptable. (It was noted that the study director was in fact [redacted] as in a 90-day rat study from the same laboratory. M. Kuwahara performed the histopathological examinations and was apparently the report writer.)
Because of the only minor effects (slightly higher blood urea nitrogen, slight caecal distention) at the mid dose level of 10000 ppm (equal to 1221 mg/kg bw/day in males) that were not accompanied by any histopathological findings, this dose is considered the NOAEL. Target organs at the very high dose of ca 6300 mg/kg bw/d (50000 ppm) were the caecum and the bladder. Clinical chemistry findings also suggest a weak effect on the liver. This dose level was clearly toxic as additionally proven by effects on body weight gain, food consumption and efficiency and on red blood cell parameters. Thus, the outcome of this study is in line or at least not in contradiction to the previous study by [redacted] (1991, TOX9552363) on CD mice in which no effects were observed up to the top dose level of 4500 mg/kg bw/day. The lower urinary pH in males in all dose groups is due to acidic properties of the test substance and cannot be considered a toxic effect.
In contrast to the publication by Chan and Mahler (1992, TOX9551954, reported below), no histological changes of the salivary glands were observed in this study as they had occurred in another strain from dietary concentrations of 6250 ppm (1065 mg/kg bw/day) onwards. Beside possible strain differences, another explanation might be that [redacted] (1995, ASB2012-11453) examined the sublingual and submaxillary glands histologically but not the parotids. In the study by [redacted] (1991, TOX9552363), histopathological examination of salivary glands was confined to the submaxillary."

Remark RMS:
The GRG is requested to submit the NTP study in rats and mice performed by Chan and Mahler (1992, TOX9551954) together with an OECD summary and an evaluation of the results (including the mechanistic study on the salivary gland).

B.6.3.2.13. Oral 13-week toxicity study in mice – study 2

Data point:	CA 5.3.2/018
Report author	[redacted]
Report year	1991
Report title	Glyphosate: 13 week dietary toxicity study in mice
Report No	7024
Document No	Not reported
Guidelines followed in study	FIFRA 82-1; OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	Ophthalmoscopy and detailed clinical observations were not performed prior to dosing. Sensory reactivity to stimuli was not performed towards the end of exposure period. Reticulocyte count, platelet count and a measure of blood clotting time/potential was not measured during the haematological examinations. Clinical biochemistry determination did not include the following parameters: HDL, LDL and urea. Serum total T4, T3 and TSH were not measured at study termination. At necropsy, the oestrus cycle of all females was not determined. Organ weight of the thyroid gland was not determined; histopathology was performed without bone/bone marrow, cervix, coagulating glands, spinal cord and vagina. In addition, individual data for weekly body weights, food consumption, water consumption are not reported. Deviations from the current version of OECD 408 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 408. Further, it should be noted that the highest dose tested (~4500 mg/kg bw/day) is far above the limit dose of 1000 mg/kg bw/day according to OECD 408.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG**: Valid, category 2a. **Conclusion AGG**: The study is considered acceptable but with restrictions (reliable with restrictions) as only a limited number of samples could be analysed for clinical chemistry due to low sample volumes.

Executive Summary
This study was designed to give toxicity information over 13 weeks on glyphosate administered to mice via the diet at concentrations calculated to achieve dose levels of 0, 200, 1000 or 4500 mg/kg bw/day. The group size was 10 animals per sex and dose group.
The animals were examined for mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry, gross pathology, organ weights and histopathology.
In conclusion, dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg glyphosate /kg bw/day produced no findings which could be directly attributed to administration of the test material.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate

Identification: Not reported
Description: White powder
Lot/Batch#: 161-JRJ-131-2 and 003-89-A
Purity: 99.5% (batch 161-JRJ-131-2) and 98.0% (batch 003-89-A)
Stability of test compound: Not reported

2. **Vehicle and/ or positive control:** Diet/ none
3. **Test animals:**

Species:	Mouse
Strain:	CD-1
Source:	[redacted]
Age:	Ca. 4 weeks
Sex:	Male and female
Weight at dosing:	♂ ca. 21 g; ♀ ca. 18 g upon arrival. ♂ 28.4 g; ♀ 21.8 g at the beginning of dosing
Acclimation period:	13 days
Diet/Food:	SDS Expanded (Fine Ground) Maintenance Diet No. 1
Water:	Tap water, *ad libitum*
Housing:	One male or one female per cage in suspended polypropylene cages (48.0 x 15.0 x 12.0 cm) with wire grid tops. Sterilised white wood shavings were used as bedding.
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 55 ± 10% Air changes: ca. 15 /hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-03-06 to 1989-06-06

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 10 CD-1 mice per sex for a minimum of 90 days. Male and female dietary concentrations were adjusted on a weekly basis to achieve an intake of 0, 200, 1000 or 4500 mg/kg bw/day.

Table 6.3.2-1: Glyphosate – 13 week dietary toxicity study in mice [redacted] 1991): Study design

Test group	Test substance intake [mg/kg bw/day]	Males	Females
Control	0	10	10
Low	200	10	10
Mid	1000	10	10
High	4500	10	10

Analysis of the test diet
Fresh diets were prepared once each week in SDS Expanded (Fine Ground) Maintenance Diet No. 1. The method of preparation was by direct admixture of test material to untreated diet and blending for 20 min in a Winkworth change drum mixer. Each mixed batch was stored in a closed container at ambient temperature.

A 100 g sample of diet from each group/sex was retained immediately after each diet preparation. In addition, usually 3 x 100 g samples were also taken for routine homogeneity and accuracy assessment from diets prepared for Weeks 1, 6 and 13. In addition, data proving homogeneity and 21-day stability of glyphosate were generated prior to the commencement of the study.

Mortality
Viability was checked once each morning and once as late as practicable each day.

Clinical observations

All animals were examined for reaction to treatment during the day. The onset, intensity and duration of these signs were recorded.

All animals received a detailed clinical examination once each week.

Body weight
The weight of each animal was recorded once during the week before the start of treatment and once each week thereafter.

Food consumption and water consumption
The quantity of food consumed by each cage of animals was recorded once each week, commencing one week before the start of treatment and once each week thereafter.

Water consumption was monitored by visual inspection throughout the treatment period.

Ophthalmoscopy examination
The eyes of all animals in the Control and High dose groups were examined using an indirect ophthalmoscope after the application of a mydriatic agent (1 % Mydriacyl). Anterior, lenticular and fundic areas were evaluated. This ophthalmoscopic examination was undertaken during Week 12 of treatment.

Haematology and clinical chemistry
Samples were taken from all rats from each group during Week 13 of dosing.
Blood samples for haematology were collected from the orbital sinus under light ether anaesthesia and for clinical chemistry via the dorsal aorta at necropsy.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, total white blood cell count, differential white blood cell count, calculations of absolute indices and Hepato Quick (clotting time) on a sample obtained by tailsnip without anaesthesia.

Clinical chemistry
The following parameters were determined: Alkaline phosphatase (AP), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), creatinine (Crea), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), glucose (Glu), total cholesterol (Chol), total bilirubin (T.Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P), plasma cholinesterase (ChE), RBC cholinesterase.

Sacrifice and pathology
All animals were killed and necropsied. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissection and necropsy were performed under the supervision of a pathologist. The premature decedents were also necropsied.

Organ weights
The following organs were weighed: Adrenals, brain, heart, kidneys, liver (with gall bladder), lungs, ovaries, pituitary, prostate, spleen, testes (with epididymides), thymus and uterus.

Histopathology
The following tissues were processed and examined histopathologically from all Control and High dose animals for the parameters listed below. Only one eye per animal was processed and examined. In addition all other animals received histopathological examination of liver, kidneys and lungs. The premature decedents also underwent a full histopathological examination. The following parameters were determined: Adrenals, aortic arch, any abnormal tissues, urinary bladder, brain, eyes, heart, intestine (duodenum, jejunum, ileum, caecum, colon), kidneys, liver (with gall bladder), lungs (perfused), mammary gland, mesenteric lymph node, muscle (thigh), nasal cavity, oesophagus, ovaries, pancreas, pituitary, prostate, sciatic nerve, seminal vesicles, skin, spleen, stomach (glandular and non-glandular), submaxillary salivary gland, submandibular lymph node, testes (with epididymides), thymus, thyroid (with parathyroid), tongue, trachea and uterus. In addition, for the following tissues samples were taken and stored: bone (sternum and rib), rectum and nasal cavity.

Statistics

Haematology, clinical chemistry, organ weight and body 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 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 in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as a Kruskal-Wallis ANOVA was used. Organ weights were also analysed conditional on body weight (i.e. analysis of covariance). Histopathology data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS

The group mean achieved dosages were in close agreement with the nominal values (93-95% of target dose). The majority of diets prepared for Weeks 1, 6 and 13 were seen to be within acceptable limits (± 10 %) for accuracy of concentration and homogeneity. In Week 1, the concentration for Group 2 (male) was -10.7 % as compared to nominal concentration and the coefficient of variation was 11.6 %. A repeat analysis for this group from diets prepared for Week 2 showed an acceptable level of concentration (+1.2 %) and coefficient of variation (1.7 %). At Week 13 the concentration for Group 3 (male and female) were -14.8 % and -14.2 % respectively and the coefficients of variation were 9.8 % and 7.1 % respectively. Archive samples for these groups from diets prepared for Week 11 were analysed and showed an acceptable level of concentration (+3.0 % for female and -2.6 % for male) and coefficient of variation (3.8 % for female and 4.8 % for male).

B. MORTALITY

There were 6 unscheduled deaths; one Control dose male (killed *in extremis* because it was unable to eat), 2 control dose females (one which died at bleed and the other which was killed *in extremis* due to its general condition), one Low dose male (which died during haematology sampling) and 2 High dose females (one of which was found dead and the other which was killed *in extremis* due to its general condition). None of these deaths could be attributed to administration of glyphosate.

C. CLINICAL OBSERVATIONS

There were no clinical signs in the control and treated groups that were considered to be due to administration of glyphosate.

D. BODY WEIGHT

There were no notable intergroup differences in either sex.

E. FOOD CONSUMPTION AND WATER CONSUMPTION

There were no notable intergroup differences in total food or water consumed in either sex at any time.

F. OPHTHALMOSCOPIC EXAMINATION

There were no notable findings in either sex.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

No notable intergroup differences were found in either sex. The statistically significant increase in Hepato Quick sampling time ($P < 0.01$), seen in the Low dose group was considered chance due to the lack of an effect in the other groups receiving glyphosate.

Table 6.3.2-2: Glyphosate – 13 week dietary toxicity study in mice [redacted], 1991): Selected haematology findings

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	200	1000	4500	0	200	1000	4500
Hepato Quick (clotting time) [s]	18 ± 2	18 ± 1	18 ± 1	18 ± 1	16.6 ± 1.1	**17.9** ± 1.0**	17.3 ± 0.7	17.1 ± 0.4

** Statistically significant from controls ($p < 0.01$)

Blood clinical chemistry
In males and females, there were no statistically significant intergroup differences.

Alkaline phosphatase and plasma cholinesterase were slightly increased with no true dose response relationship and potassium was decreased in males in all dose groups. It was noted that glucose tended to increase with increasing dose level of glyphosate in males.

Glucose and alkaline phosphatase were slightly increased in females in all groups, however a dose response relationship was absent.

Limited sample volume precluded analysis of total protein, albumin and cholesterol. The number of samples available for analysis of some other parameters was also affected by the small volume of plasma obtained from many animals (sodium, potassium, chloride was measured in limited number of males and females).

Table 6.3.2-1: Glyphosate – 13 week dietary toxicity study in mice [redacted], 1991): Selected clinical chemistry findings

Parameter	Dose group [mg/kg bw/day]							
	Males				Females			
	0	200	1000	4500	0	200	1000	4500
Alkaline phosphatase (AP) [IU/L]	88 ± 25	111 ± 29	143 ± 79	139 ± 30	135 ± 39	173 ± 36	159 ± 46	178 ± 41
Plasma cholinesterase (ChE) [IU/L]	5843 ± 548	6821 ± 847	6919 ± 1208	6789 ± 849	11125 ± 1195	9900 ± 630	10599 ± 1302	10232 ± 1860
RBC cholinesterase (RChE) [IU/L]	1413 ± 936	1213 ± 369	1177 ± 504	1052 ± 481	1211 ± 426	1229 ± 276	1288 ± 684	2033 ± 971
Glucose [mmol/L]	14.7 ± 5.72	15.54 ± 4.05	16.12 ± 3.37	18.17 ± 5.86	14.57 ± 5.20	16.63 ± 6.02	15.01 ± 4.79	16.63 ± 4.54
Potassium [mmol/L]	13.2 ± 1.7	13.1 ± 1.0	12.8 ± 1.8	12.6 ± 2.1	12.4 ± 3.6	13.0 ± 2.0	11.7 ± 2.8	11.1 ± 2.1

G. NECROPSY

Organ weights
There were no notable intergroup differences in either sex.

The increase in testes weight in the male Low and Intermediate dose groups ($P < 0.01$), seen in absolute values and after final adjustment for body weight was considered to be chance, due to the lack of effect in the High dose group. The increases in thymus and prostate weight seen in the male Intermediate dose group only were also considered to be chance.

The increase in absolute pituitary weight in the female High dose group ($P < 0.05$) was considered to be chance, due to the lack of dose response relationship and the variability seen the other dose groups.

Table 6.3.2-4: Glyphosate – 13 week dietary toxicity study in mice [redacted], 1991): Selected organ weights

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	200	1000	4500	0	200	1000	4500
Pituitary	Absolute organ weight [g]	0.00111 ± 0.00033	0.00100 ± 0	0.00120 ± 0.00042	0.00100 ± 0	0.0013 ± 0.0005	0.0017 ± 0.0008	0.0011 ± 0.0003	**0.0019** ± 0.0006*
Prostate	Absolute organ weight [g]	0.021 ± 0.008	0.019 ± 0.011	0.032 ± 0.015	0.020 ± 0.005	-	-	-	-
Testes	Absolute organ weight [g]	0.34 ± 0.05	**0.39** ± 0.02 **	**0.38** ± 0.03 **	0.34 ± 0.03	-	-	-	-
Thymus	Absolute organ weight [g]	0.017 ± 0.005	0.021 ± 0.003	**0.024** ± 0.008 **	0.017 ± 0.005	0.023 ± 0.005	0.025 ± 0.008	0.024 ± 0.007	0.030 ± 0.011

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Gross pathology
There were no findings which could be related to treatment with Glyphosate.

Histopathology
There was a decrease in diffuse vacuolation observed in the liver in the control (males: 4/10; females: 1/10), Low dose (males: 2/10) and Intermediate dose (females: 3/10) animals.

An increase in cortical tubular epithelial hypertrophy in kidney was seen in Intermediate dose females (5/10), where only 1/10 controls and 2/10 Low dose animals showed the findings, but no High dose females. There was also a marginal increase seen in High dose males (4/10), where 2/10 controls, 0/10 Low dose and 2/10 Intermediate dose animals had the finding. The significance of this finding is doubtful in view of its presence in controls and the lack of true dose response relationship.

There were also a number of histopathological changes usually seen in rats of this age and strain at [redacted] many of these being of inflammatory nature, and considered to be unrelated to treatment with Glyphosate.

Table 6.3.2-5: Glyphosate – 13 week dietary toxicity study in mice [redacted], 1991): Selected histopathological findings (incidence)

Parameter		Dose group [mg/kg bw/day]							
		Males				Females			
		0	200	1000	4500	0	200	1000	4500
Liver	No abnormalities detected	2	0	2	5	3	2	1	2
	Centrilobular vacuolation	2	2	4	1	2	3	1	3
	Diffuse vacuolation	4	2	0	0	1	0	3	0
Kidneys	No abnormalities detected	6	4	3	2	5	6	3	6
	Cortical tubular epithelial hypertrophy	2	0	2	4	1	2	5	0

III. CONCLUSIONS

This study was designed to give toxicity information over 13 weeks on glyphosate administered to mice via the diet at concentrations calculated to achieve dose levels of 0, 200, 1000 or 4500 mg/kg bw/day.

The animals were examined for mortality, clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry, gross pathology, organ weights and histopathology.

In conclusion, dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg glyphosate /kg bw/day produced no findings which could be directly attributed to administration of the test material. The highest dose of 4500 mg/kg bw/day was considered the NOEL in this study by the study authors.

Assessment and conclusion by applicant:
In this study, groups of male and female CD-1 mice were administered glyphosate in the diet at dose levels of 0, 200, 1000 or 4500 mg/kg bw/day for 13 weeks according to OECD 408 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Dosing CD-1 mice via the diet for 13 weeks with up to and including 4500 mg/kg bw/day glyphosate produced no findings which could be directly attributed to administration of the test material.

The highest dose of 4500 mg/kg bw/day is considered the NOAEL in this study, although evaluation of clinical chemistry parameters was of limited scientific value only.

Assessment and conclusion by RMS:
The RMS considers this study as acceptable but with restrictions (reliable with restrictions) due to limited number of animals included from some clinical chemistry parameters. The deviations compared with the current OECD 408 (2018) are due to the fact that the study was aligned to an older version of OECD TG 408.

The RMS agrees with the derived NOAEL of 4500 mg/kg bw/day (highest dose tested), although it should be noted that evaluation of clinical chemistry parameters was of limited scientific value only. This conclusion is in agreement with the original evaluation of the study in the DAR and RAR (2015).

In this study no histopathological changes were noted in the salivary gland, however, only the submaxillary gland was investigated and not the sublingual or parotid gland.

B.6.3.2.14. Oral 13-week toxicity study in mice – study 3

Data point	CA 5.3.2/019
Report author	[redacted]
Report year	1979
Report title	A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice
Report No	77-2111
Document No	Not reported
Guidelines followed in study	No guideline followed, similar to OECD 408 (1981)
Deviations from current test guideline (OECD 408, 2018)	No sensory reactivity was investigated; no ophthalmoscopy was performed; no haematology or clinical chemistry was performed; organ weights of the adrenals, epididymides, prostate and seminal vesicles and coagulating glands, pituitary gland, thyroid, thymus and uterus were not determined; histopathology was performed without aorta, coagulating glands, mammary glands, seminal vesicles, skin, trachea and vagina.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (pre-GLP).
Acceptability/Reliability	**Conclusion GRG**: Supportive, category 3b **Conclusion AGG**: The study was performed according to a testing regime similar to OECD 408 (1981). Although the main deficiency was that

	haematological and clinical chemistry parameters were not included, overall the study was well performed. Therefore, this study is considered as acceptable but with restrictions (reliable with restrictions).

Executive Summary

A total of 120 CD-1 mice were randomly distributed into four groups of 15 animals per sex and group. The test material, glyphosate (Roundup® Technical), was administered to the animals in Groups II (low), III (mid) and IV (high) at dose levels of 5000, 10000 or 50000 ppm (equivalent to 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females) via dietary admixture for three months. Control animals (Group I) received untreated diet.

Animals were observed for mortality, clinical signs, body weight changes, food consumption, organ weight changes and gross pathology and histopathological evaluation of selected tissues was performed on 10 animals per sex and group from the control and high-dose group.

Body weight gain of the high-dose males and females was lower than that of the control animals after 13 weeks of test substance administration. In addition, body weight was decreased at several time points in high-dose males and females. These effects on body weight are considered treatment-related and adverse by the RMS as the changes are >10% compared with the controls. Food consumption of the low-, mid- and high-dose males and females was generally greater than that of control animals throughout the period of test substance administration. All other parameters evaluated (mortality, physical observations, organ weights, organ/body weight ratios and organ/brain weight ratios) were considered to be either comparable between treated and control animals or of no toxicological significance.

The results of gross *post mortem* examinations as well as the histopathologic evaluations of selected tissues did not reveal any evidence of effects related to the administration of glyphosate (Roundup® Technical).

The RMS proposes a NOAEL of 10000 ppm (equivalent to 1867.2 and 2734.7 mg/kg bw/day in males and females, respectively). However, it should be noted that no haematological and clinical chemistry parameters were included in this study and therefore this NOAEL is of limited value.

I. MATERIALS AND METHODS

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 / none

3. Test animals:

Species:	Mouse
Strain:	CD-1, COBS (ICR derived)
Source:	[redacted]
Age:	41 days
Sex:	Male and female
Weight at dosing:	♂ 28 g (23 – 33 g); ♀ 22 g (19 – 25 g)

Acclimation period:	13 days	
Diet/Food:	Standard laboratory diet (Purina Laboratory Chow, No. 5001®) *ad libitum*; fresh food presented weekly.	
Water:	Automated water system (Elizabethtown Water Company), *ad libitum*	
Housing:	Individually in elevated stainless steel wire mesh cages.	
Environmental conditions:	Temperature:	20.6 – 28.3 °C
	Humidity:	Not reported
	Air changes:	Not reported
		12 hour dark/light cycle

B: Study design and methods

In life dates: 1979-05-21 to 1979-08-21

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 15 CD-1 mice per sex for 92 – 93 days. Dietary concentrations were 0, 5000, 10000 or 50000 ppm (equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females).

Table 6.3.2-1: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice [redacted] 1979): Study design

Test group	Dietary concentration [ppm]	Compound intake [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	15	15
Low	5000	♂: 944.1; ♀: 1527.7	15	15
Mid	10000	♂: 1867.2; ♀: 2734.7	15	15
High	50000	♂: 9707.0; ♀: 14858.2	15	15

Analysis of the test diet
Prior to initiation of the study, full sized batches of diets were prepared at dietary levels of 5000, 10000 and 50000 ppm. Duplicate samples of approximately 50 g each were taken from the right and left of the top, middle and bottom of the mixer (12 samples/batch) and sent frozen to the sponsor (1979-04-30) for analysis of homogeneity. In addition, approximate 25 g duplicate samples were taken from the feed buckets and stored frozen on Days 0, 1, 2, 5, 7 and 14. On Day 14, these samples were sent frozen to the sponsor for analytical confirmation of stability (1979-05-14). Additional samples were taken throughout the study, frozen and half of them sent to sponsor for analysis.

Mortality
Viability was checked twice daily.

Clinical observations
All animals were examined for gross signs of toxicological or pharmacological effects twice daily.
Additionally, all animals received a detailed physical examination for signs of local or systemic toxicity and pharmacologic effects once each week.

Body weight
The weight of each animal was recorded twice prior to treatment, weekly during treatment and terminally (after fasting).

Food consumption and compound intake
The quantity of food consumed by each animal was recorded once prior to treatment and weekly during treatment. The test substance intake was calculated from food consumption data. Please refer to the table above for information on the compound intake.

Sacrifice and pathology

All animals dying spontaneously, killed in a moribund condition and all survivors killed by exsanguination from the abdominal aorta under ether anaesthesia were subjected to necropsy.
A complete gross post-mortem examination was performed on all animals.

Organ weights
The following organs were weighed: Brain, heart, kidneys, liver, ovaries, spleen and testes.
Organ/body weight and organ/brain weight ratios were calculated.

Histopathology
Histopathological examinations of the following organs and tissues were made on sections from 10 control and 10 high dose animals stained with haematoxylin and eosin: Adrenals, bone and bone marrow, blood smear, brain, epididymides, eyes, gall bladder, gross lesions, heart, intestine (duodenum, jejunum, ileum, caecum, colon), kidneys, liver, lungs, lymph node, muscle, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland (mandibular), sciatic nerve, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, urinary bladder, uterus (with cervix), gross lesions, tissue masses or suspect tumours and regional lymph nodes.

Statistics
Body weight, food consumption, organ weights, organ/body weight 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 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 was 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 l%, two-sided risk level. All other statistical tests were conducted at the 5% and l%, two-sided risk levels.

II. RESULTS AND DISCUSSION

A. ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS
The analytical results show that glyphosate is stable in the feed mixture for the one-week period required (7 days after mixing, mean concentration 99.9% of target), that the mixing equipment and procedures used produce acceptably uniform feed mixtures (mean concentration 97.2% of target) and that proper glyphosate concentrations were maintained in the prepared feed diets throughout the term of the study (mean concentration 100-100.4% of target).

B. MORTALITY
One control male and one mid-dose female died spontaneously during the course of the study. All other animals survived the duration of the study.

C. CLINICAL OBSERVATIONS
Daily and weekly physical observations of the males and females in all treatment groups were of the type commonly seen in the laboratory mouse and were considered comparable to those of the control animals. No pharmacological or toxicological signs or symptoms were observed which were considered related to the administration of the test substance.

D. BODY WEIGHT
Mean body weight values of the low-, mid- and high-dose males and females were generally slightly or statistically significantly lower than control values at the second pre-test interval and throughout the course of the study. However, body weight gains of the low- and mid-dose males and females were considered comparable to those of control animals. The body weight gain of the high-dose males (+8.13 g) and females (+7.2 g) was lower than that of the control animals (+10.67 g males and +8.74 g females) after 13 weeks of test substance administration.

Table 6.3.2-2: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice ([redacted], 1979): Summary of body weight data [g]

Week	Dose group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000
-1	26.47 ± 0.70	↓25.87 ± 0.63	↓26.13 ± 0.60	↓25.73 ± 0.65	20.73 ± 0.42	↓20.67 ± 0.30	↓20.47 ± 0.31	↑20.80 ± 0.37
0	29.33 ± 0.47	↓27.67 ± 0.49	**↓27.27* ± 0.58**	**↓27.47* ± 0.54**	23.13 ± 0.35	↓22.20 ± 0.43	↓21.93 ± 0.33	↓22.00 ± 0.40
1	31.60 ± 0.55	↓31.33 ± 0.61	↓30.27 ± 0.61	**↓28.80** ± 0.48**	25.73 ± 0.37	↓24.93 ± 0.38	↓24.60 ± 0.38	**↓23.93* ± 0.43**
2	33.27 ± 0.52	↓31.47 ± 0.58	**↓31.33* ± 0.61**	**↓30.67* ± 0.49**	26.40 ± 0.42	**↓23.87** ± 0.32**	↓25.13 ± 0.43	**↓24.00** ± 0.51**
3	33.47 ± 0.62	↑33.60 ± 0.69	↓32.80 ± 0.60	**↓29.67** ± 0.49 (-11%)**	27.47 ± 0.42	**↓25.13** ± 0.51**	**↓25.87* ± 0.41**	**↓25.60* ± 0.46**
4	35.27 ± 0.67	↓34.60 ± 0.68	↓34.80 ± 0.63	**↓31.67** ± 0.52 (-10%)**	28.00 ± 0.54	↓26.40 ± 0.59	↓27.27 ± 0.42	**↓25.80* ± 0.46**
5	35.33 ± 0.64	↓33.87 ± 0.65	↓34.40 ± 0.65	**↓32.47* ± 0.50**	29.20 ± 0.54	**↓26.93** ± 0.56**	↓28.13 ± 0.41	**↓27.00** ± 0.48**
6	34.87 ± 0.66	↓33.80 ± 0.67	↓34.53 ± 0.59	**↓31.80** ± 0.59**	28.53 ± 0.55	↑28.60 ± 0.62	↓28.13 ± 0.40	**↓25.40** ± 0.43 (-11%)**
7	35.80 ± 0.68	↓35.60 ± 0.56	↓35.47 ± 0.62	**↓33.27* ± 0.67**	28.87 ± 0.52	↓27.00 ± 0.56	↓28.33 ± 0.39	↓27.93 ± 0.48
8	36.07 ± 1.13	↑36.13 ± 0.64	↑36.20 ± 0.56	↓33.53 ± 0.62	30.20 ± 0.56	**↓27.93** ± 0.58**	↓29.53 ± 0.38	**↓28.33* ± 0.40**
9	35.93 ± 1.25	↓35.47 ± 0.68	↑36.07 ± 0.57	**↓33.53§ ± 0.60**	30.07 ± 0.57	↓28.87 ± 0.58	↓29.67 ± 0.43	↓28.73 ± 0.36
10	35.87 ± 1.38	↑36.20 ± 0.64	↑37.33 ± 0.58	↓35.07 ± 0.63	30.13 ± 0.52	↓28.53 ± 0.65	↑30.20 ± 0.43	↓28.87 ± 0.38
11	37.50 ± 0.82	↓36.67 ± 0.66	↑37.53 ± 0.60	**↓34.87* ± 0.68**	30.00 ± 0.68	**↓27.20* ± 0.69**	↓28.53 ± 0.43	↓28.20 ± 0.48
12	37.21 ± 0.81	↓36.27 ± 0.64	↓36.60 ± 0.61	**↓34.07* ± 0.79**	30.40 ± 0.65	**↓27.80** ± 0.57**	↑30.93 ± 0.56	**↓28.47* ± 0.46**
13	40.00 ± 0.89	↓39.40 ± 0.57	↓37.93 ± 0.68	**↓35.60** ± 0.76 (-11%)**	31.87 ± 0.62	↓31.00 ± 0.62	**↓30.64* ± 0.44**	**↓29.20** ± 0.45 (-8%)**
Body weight gain week 0 to 13	10.67	11.73	10.66	8.13 (-24%)	8.74	8.80	8.71	7.20 (-18%)

* 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 test; $p \leq 0.05$)

E. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption of the low-, mid- and high-dose males and females was generally slightly or significantly greater than that of control animals throughout the period of test substance administration.

Table 6.3.2-3: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice [redacted] 1979): Summary of food consumption data [g/kg bw/day]

Week	Dose group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000
0	188.98 ± 9.10	↑220.81 ± 15.65	↑224 58 ± 13.77	↑214.18 ± 19.66	280.73 ± 28.16	↑357.58 ± 26.50	↑314.42 ± 31.31	↑339.16 ± 28.38
1	186.59 ± 8.47	↑192.70 ± 7.59	↑203 20 ± 8.74	**↑240.60** ** **± 16.43**	236.17 ± 12.25	**↑287.68** * **± 12.69**	↑245.93 ± 16.75	**↑355.83** ** **± 6.99**
2	185.66 ± 4.87	↑222.49 ± 19.73	**↑247.86** §§ **± 11.80**	↑207.33 ± 17.97	269.50 ± 17.10	**↑403 54** * **± 35.71**	↑311.22 ± 24.80	↑364.73 ± 34.23
3	154.75 ± 14.57	**↑217.73** * **± 20.71**	↑206.49 ± 11.11	↑200.07 ± 16.86	261.13 ± 23.33	↑352.47 ± 32.42	↑348.84 ± 33.78	↑322.33 ± 35.80
4	171.69 ± 6.79	↑182.35 ± 5.89	**↑239.02** ** **± 11.00**	**↑207.30** * **± 10.97**	260.40 ± 16.33	↑279.24 ± 17.16	↑275.37 ± 18.53	↑269.39 ± 22.77
5	168.81 ± 8.76	↑209.64 ± 15.63	↑197 34 ± 12.57	↑186.35 ± 7.54	256.47 ± 20.22	↑314.80 ± 29.42	↑260.91 ± 26.79	↑286.65 ± 38.37
6	168.40 ± 8.29	↑215.74 ± 16.94	↑189.12 ± 10.47	↑205.03 ± 17.19	297.49 ± 26.48	↑327.30 ± 20.84	↓277.75 ± 25.63	↓272.62 ± 24.82
7	164.12 ± 5.46	↑185.65 ± 8.17	↑173.75 ± 7.47	↑204.51 ± 15.53	248.79 ± 11.55	↓248.75 ± 9.40	↑252.13 ± 13.61	↑252.93 ± 15.06
8	137.01 ± 10.32	**↑182 55** * **± 13.83**	↑173.69 ± 8.37	↑173.22 ± 10.92	286.13 ± 29.16	↑286.84 ± 25.42	↓254.55 ± 24.12	↓282.57 ± 31.90
9	140.61 ± 5.50	↑174.21 ± 16.81	↑170 99 ± 12.12	↑162.61 ± 10.70	316.03 ± 53.33	↑363.19 ± 46.71	↓289.64 ± 38.76	↑346.02 ± 46.25
10	150.61 ± 8.15	↑174.77 ± 9.40	↑155.69 ± 6.42	↑175.50 ± 13.52	226.59 ± 25.29	↑265.17 ± 26.31	↑252.99 ± 25.28	↑233.77 ± 27.80
11	136.76 ± 4.94	↑175.35 ± 15.23	↑154 51 ± 5.88	**↑183.55** § **± 12.34**	270.78 ± 26.43	↑293.56 ± 35.37	↓262.94 ± 22.68	↑288.22 ± 37.41
12	151.93 ± 6.42	↑176.09 ± 10.42	↑170 28 ± 8.13	↑188.33 ± 15.03	262.66 ± 23.70	↑293.35 ± 29.76	↑268.41 ± 22.33	↑267.07 ± 26.18
13	126.14 ± 5.71	↑155.19 ± 13.93	**↑156.22** § **± 6.82**	**↑198.71** §§ **± 15.47**	253.21 ± 23.45	↑269.36 ± 26.04	↑266.01 ± 23.29	↑333.23 ± 32.54

* 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 test; $p \leq 0.05$);
§§ Significantly different from control (Dunn's Rank Sum test; $p \leq 0.01$)

CD-1 mice received dose levels of 0, 5000, 10000 or 50000 ppm which when applied to the body weight and feed consumption was equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females.

F. NECROPSY

Gross pathology
Macroscopic post mortem observations did not reveal any changes considered related to the administration of test material.

Organ weights
The mean organ/body weight ratios of the brain, heart, kidneys and liver of the high-dose males and the mean relative liver weight of the mid-dose males, were significantly greater than those of the control values. These differences were probably reflective of the slightly lower terminal body weights of the mid- and high-dose males, respectively. All other mean organ weights and organ/body weight ratios were considered comparable between the control and treated males and females.

There were no significant differences in mean absolute organ weights and organ/brain weight ratios between the controls and the low-, mid- and high-dose males and females.

Table 6.3.2-4: A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice ([redacted] 1979): Organ weight data and organ/body weight ratios

	Dose group [ppm]							
	Males				Females			
	0	5000	10000	50000	0	5000	10000	50000
Body weight [g]	40.0 ± 0.9	↓39.6 ± 0.6	↓37.9 ± 0.7	↓**35.6**** ± **0.8**	26.4 ± 0.5	↑26.5 ± 0.5	↑26.8 ± 0.4	↓26.0 ± 0.3
Brain [g]	0.459 ± 0.004	↓0.449 ± 0.007	↓0.455 ± 0.007	↓0.458 ± 0.009	0.455 ± 0.007	↑0.467 ± 0.007	↑0.466 ± 0.007	0.455 ± 0.006
Brain [%]	1.154 ± 0.024	↓1.139 ± 0.027	↑1.206 ± 0.030	↑**1.291**** ± **0.027**	1.728 ± 0.037	↑1.765 ± 0.037	↑1.745 ± 0.033	↑1.754 ± 0.029
Gonads [g]	0.3471 ± 0.0104	↑0.3561 ± 0.0346	↑0.3653 ± 0.0295	↓0.3219 ± 0.0083	0.0161 ± 0.0015	↑0.0262 ± 0.0096	↑0.0185 ± 0.0016	↓0.0150 ± 0.0012
Gonads [%]	0.8715 ± 0.0284	↑0.9008 ± 0.0885	↑0.9666 ± 0.0788	↑0.9096 ± 0.0297	0.0608 ± 0.0055	↑0.1008 ± 0.0386	↑0.0688 ± 0.0057	↓0.0574 ± 0.0043
Heart [g]	0.148 ± 0.003	↑0.150 ± 0.006	↑0.152 ± 0.005	↑0.153 ± 0.006	0.132 ± 0.004	↓0.128 ± 0.004	↑0.142 ± 0.008	↓0.126 ± 0.004
Heart [%]	0.3719 ± 0.0118	↑0.3796 ± 0.0143	↑0.4010 ± 0.0107	↑**0.4300*** ± **0.0159**	0.5014 ± 0.0126	↓0.4856 ± 0.0151	↑0.5324 ± 0.0274	↓0.4845 ± 0.1189
Spleen [g]	0.0754 ± 0.0039	↓0.0721 ± 0.0027	↓0.0723 ± 0.0028	↓0.0644 ± 0.0036	0.0916 ± 0.0073	↑0.0933 ± 0.0035	↑0.0951 ± 0.0046	↓0.0857 ± 0.0039
Spleen [%]	0.1901 ± 0.0118	↓0.1820 ± 0.0059	↑0.1918 ± 0.0087	↓0.1818 ± 0.0109	0.3448 ± 0.0246	↑0.3526 ± 0.0143	↑0.3553 ± 0.0159	↓0.3296 ± 0.0134
Kidneys [g]	0.535 ± 0.016	0.535 ± 0.017	↑0.545 ± 0.010	↑0.566 ± 0.016	0.383 ± 0.011	↑0.387 ± 0.012	↑0.387 ± 0.014	↓0.376 ± 0.006
Kidneys [%]	1.342 ± 0.040	↑1.357 ± 0.052	↑1.441 ± 0.027	↑**1.590**** ± **0.032**	1.450 ± 0.028	↑1.463 ± 0.052	↓1.448 ± 0.044	↓1.448 ± 0.024
Liver [g]	1.333 ± 0.039	↓1.331 ± 0.028	↑1.377 ± 0.026	↓1.329 ± 0.034	1.133 ± 0.028	↓1.115 ± 0.024	↓1.106 ± 0.036	↓1.068 ± 0.030
Liver [%]	3.334 ± 0.069	↑3.365 ± 0.069	↑**3.637* ± 0.066**	↑**3.737**** ± **0.076**	4.293 ± 0.083	↓4.207 ± 0.077	↓4.133 ± 0.116	↓4.104 ± 0.098

* Significantly different from control (Dunnett's test; $p \leq 0.05$);
** Significantly different from control (Dunnett's test; $p \leq 0.01$)

Gross necropsy / histopathology
Results of the gross *post mortem* examinations performed on all animals as well as histopathological evaluations of selected tissues from 10 animals/sex/group in the control and high-dose groups did not reveal any evidence of effects which could be attributed to the administration of glyphosate (Roundup® Technical).

III. CONCLUSIONS

Body weight gain of the high-dose males and females was lower than that of the control animals after 13 weeks of test substance administration. Food consumption of the low-, mid- and high-dose males and females was generally greater than that of control animals throughout the period of test substance administration. All other parameters evaluated (mortality, physical observations, organ weights, organ/body weight ratios and organ/brain weight ratios) were considered to be either comparable between treated and control animals or of no toxicological significance.

The results of gross *post mortem* examinations as well as the histopathologic evaluations of selected tissues did not reveal any evidence of effects related to the administration of glyphosate (Roundup® Technical).

Assessment and conclusion by applicant:
In this study, the test material glyphosate was administered to groups of CD-1 mice at dose levels of 0, 5000, 10000 or 50000 ppm (equivalent to 0, 944.1, 1867.2 or 9707.0 mg/kg bw/day for males and 0, 1527.7, 2734.7 or 14858.2 mg/kg bw/day for females) via the diet for three months.

The study was conducted according to a testing regime similar to OECD 408 (1981) at a time when GLP was not compulsory. However, there were major deviations to current standards: no sensory reactivity tests were performed; no haematology/clinical chemistry were performed; organ weights of the adrenals, epididymides, prostate + seminal vesicles and coagulating glands, pituitary gland, thyroid, thymus and uterus were not determined; histopathology was performed without aorta, coagulating glands, mammary glands, seminal vesicles, skin, trachea and vagina.

Apart from these major deviations, the study was well conducted and can provide supplemental information for the assessment of repeated dose toxicity of glyphosate. The study is therefore considered to be only supplementary.

All in-life data (physical observations, body weight and food consumption) and gross necropsy, organ weights and histopathology observations indicated no adverse effects of glyphosate at any of the dose levels administered. Therefore, under the conditions of this study, the NOAEL for oral administration in the diet in CD mice can be set at the highest dose of 50000 ppm (equivalent to approx. 9707.0 and 14858.2 mg/kg bw/day in male and female mice, respectively).

Assessment and conclusion by RMS:
In contrast to the applicant and in contrast to the previous assessment, the RMS considers this study as acceptable but with restrictions (reliable with restrictions) instead of supplementary. Despite the main deficiency that haematological and clinical chemistry parameters were not included, the study was overall well performed and was according to a testing regime similar to OECD 408 (1981)

The RMS disagrees with the NOAEL proposed by the notifier at the highest dose tested. The RMS considers the body weight effects, although not significant, at the top dose as adverse and treatment-related. Body weight gain from week 0 to 13 was decreased in both males and females (-24% and -18% compared with controls at the top dose, respectively) at the top dose of 50000 ppm. In addition, body weight was also decreased by more than 10% in males at week 3, week 4 and week 13 and in females at week 6 compared with controls. There were no other adverse findings. However, it should be noted that no haematological and clinical chemistry parameters were included in this study and therefore this NOAEL is of limited value.

The NOAEL of 10000 ppm is in agreement with the original conclusion from the DAR. In the RAR (2015) this study was not considered acceptable and not included in the assessment.

B.6.3.2.15. Oral 13-week toxicity study in dogs – study 1

Data point	CA 5.3.2/020
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs
Report No	29646[redacted]
Document No	Not reported
Guidelines followed in study	OECD 409 (1998); JMMAF 12 NohSan No. 8147
Deviations from current test guideline (OECD 409, 1998)	None
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary
Groups of four Beagle dogs per sex received the test item, glyphosate technical, by daily administration (capsule) at dose-levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks. The duration of the treatment period for the high-dose group was shortened to 11 weeks for ethical reasons due to marked toxic effects.
The animals were checked daily for mortality and clinical signs. Body weight was recorded weekly. Food consumption was estimated daily. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations, as well as urinalysis, were performed before the beginning of the treatment period, in Week 7 and at the end of the treatment period. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.

In the low- and mid-dose groups no treatment-related signs were noted. No haematological, blood biochemical, urinary or histopathological effects were observed. Only a slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.
At 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) or body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.

Laboratory investigations in the surviving animals demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females).

Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day. These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused either directly or indirectly, by the test item. Further major microscopic changes in moribund sacrificed animals were found in the kidneys (bilateral vacuolation of cortical tubules, sometimes with pigment deposits), liver (diffuse macrovesicular vacuolation, acute inflammation and/or pigment deposits), oesophagus, lung, uterus (atrophy) and/or bone marrow (increased number of adipocytes). These findings were associated with numerous changes in laboratory parameters (haemoconcentration, increased urea and creatinine levels, decreased urea, protein, albumin and bilirubin levels and decreased liver enzyme activities).

In males treated at 300 mg/kg bw/day, an increased absolute (+18%) and relative (+25%) adrenal weight was noted. This was also seen in males treated at 1000 mg/kg bw/day. However, as the changes in adrenal weight were not accompanied by histological changes and were also not seen in females, this finding is not considered adverse.

The NOAEL is set at 300 mg/kg bw/day. At the top dose level of 1000 mg/kg bw/day, the MTD was clearly exceeded. At this dose level, clinical signs were observed (liquid/soft faeces, dehydration, vomiting) which lead to early sacrifice of two moribund animals and making termination of high dose groups after 11 weeks necessary. Further, a decreased body weight, body weight gain and food consumption was observed in both sexes, clinical chemistry and urine parameters were altered, prostate and uterus atrophy was seen and histological lesions in many organs (such as kidney liver, bone marrow) related to the moribund state of the dogs.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical
Description:	White crystalline powder
Lot/Batch#:	H05H016A
Purity:	95.7%
Stability of test compound:	Stable under storage conditions (< 30°C), light protected; Expiry date: 2008-03-25

2. **Vehicle and/ or positive control:** Empty gelatine capsules, size 12 (Torpac, NY, US)
3. **Test animals:**

Species:	Dogs
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.5 – 8.0 kg; ♀ 6.6 – 7.7 kg
Acclimation period:	14 days
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinary-sur-Orge, France), 300 g per day (Following reduced food consumption among some animals standard tinned dog food was distributed instead or in addition.)
Water:	Tap water, *ad libitum*
Housing:	Individual housing in pens containing wood shavings.
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20% Air changes: 12/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-06-08 to 2005-09-22

Animal assignment and treatment:
In a 13-week oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 300 or 1000 mg/kg bw/day glyphosate technical by capsule application. Due to marked toxic effects among animals in the group treated at 1000 mg/kg bw/day, treatment of these animals was discontinued after day 75 and the animals were sacrificed in week 11. All examinations scheduled at the end of the treatment period (ophthalmology and laboratory investigations) were carried out in week 11 for the surviving animals in this group.
The test item capsules were prepared weekly and delivered daily to the animal room, protected from light. As the test item was administered via capsules, no chemical analysis was performed during the study. The purity, characteristics and identification of the test item were indicated on the certificate of analysis that accompanied the test item.

Mortality
Each animal was checked for mortality or signs of morbidity twice a day during the treatment period, including weekends and public holidays.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals. In addition, a detailed clinical examination was performed at least once before the beginning of the treatment period and then once a week until the end of the study.

Body weight
The body weight of each animal was recorded twice before group allocation, on the first day of treatment, and then once a week until the end of the study. In addition, the group 4 animals were weighed before final sacrifice on day 75 (early sacrifice of remaining top dose animals).

Food consumption
The quantity of food consumed was recorded for each animal. Food intake per animal and per day was calculated for 7 days before the beginning of the treatment period and then throughout the study.

Ophthalmoscopic examination
Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on all animals from each test and control group before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3).

Prior to blood sampling the animals were deprived of food for an overnight period of at least 14 hours.
The following parameters were determined: Erythrocytes, haemoglobin, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes, leucocytes, differential white cell count including morphology, reticulocytes, prothrombin time, activated partial thromboplastin time, sodium, potassium, chloride, calcium, inorganic phosphorous, glucose, urea, creatinine, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and gamma-glutamyl transferase (GGT).

Urinalysis
Urine samples were collected from all animals of the test and control groups before the beginning of the treatment period, in Week 7 and at the end of the treatment period (Week 11 for Group 4 and Week 13 for Groups 1 to 3). During urine collection, the animals were deprived of food for an overnight period of at least 14 hours. The following parameters were assessed: Appearance, colour, volume, pH, specific gravity, proteins, glucose, ketones, bilirubin, nitrites, blood, urobilinogen and sediment.

Sacrifice and pathology
On completion of the treatment period (Week 11 or 13), after at least 14 hours fasting, all surviving animals were subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, thyroids with parathyroid and uterus.

Tissue samples were taken from the following organs and preserved in buffered formalin: Adrenals, aorta, bone & bone marrow (sternum and femur), brain (at three levels), caecum, colon, duodenum, epididymides, oesophagus, eyes, gall bladder, heart, ileum (with Peyer's patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostrate, rectum, salivary glands (parotid and submandibular), sciatic nerve, skin, spinal cord (cervical, thoracic and lumbar), spleen, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, ureters, urinary bladder, uterus (horns and cervix) and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY

Two unscheduled sacrifices (one male and one female) were occurred in animals given 1000 mg/kg bw/day: One male was sacrificed on Day 61 on humane grounds. Vomiting was seen once in Week 7 (before dosing) and liquid faeces were noted on many occasions in Weeks 8 and 9. Prior to sacrifice, signs of poor clinical condition including thin appearance, dehydration, and pallor of lip mucosa, coldness to the touch, hypothermia (34 – 35 °C) and hypo-activity were observed. These signs were associated with a body weight loss between Weeks 7 and 9 (-34 %) and reduced food consumption from Week 7 (generally only 25 – 50 % of this animal's daily ration was consumed), followed by an absence of food intake on the day before death. Medical care (Smecta® and Lactate Ringer®) was given in order to stop the diarrhoea and rehydrate the animal.

One female was sacrificed on Day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from Week 4 and dehydration from Week 9. Vomiting was observed once in Week 10. These signs were accompanied by a body weight loss between Weeks 8 and 11 (-22%) and decreased food consumption from Week 8 (generally only 25 – 50% of this animal's daily ration was consumed), followed by an absence of food intake on the two days prior to sacrifice. Medical care (Smecta® and lactate Ringer®) was given in many occasions.

B. CLINICAL OBSERVATIONS

No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw/day.

The following treatment-related clinical signs were reported in animals given 1000 mg/kg bw/day (excluding those killed in extremis, which are discussed separately above):

- liquid or soft faeces on several occasions in all animals,
- vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment,
- thin appearance in 1/3 males and all females,
- dehydration in 1/3 males and 2/3 females,
- pallor of ears and mouth in 1/3 females.

C. BODY WEIGHT

No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw/day during the treatment period.

Due to numerous individual body weight losses recorded from Week 4 in males and from Week 1 in females, a marked lower mean body weight was noted in animals given 1000 mg/kg bw/day at termination.

At the end of the treatment period this resulted in only a slight mean body weight gain in males (+7% vs. +31% in controls) and a mean body weight loss in females (-7% vs. +14% in controls) when compared to their body weight on Day 1. This effect on body weight was considered treatment-related (see table below).

Table 6.3.2-1: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted] 2007): Group mean weekly body weights

Time point	Mean body weight and body weight change [kg]						
	Day 1	Week 5	Week 9	Week 11	Change week 1-11	Week 13	Change week 1-13
Dose [mg/kg bw/day]	**Males**						
0	7.4 ± 0.24	9.0 ± 0.29	9.5 ± 0.25	9.7 ± 0.25	+2.3	10.4 ± 0.30	+3.0
30	↓7.2 ± 0.57	↓8.5 ± 0.52	↓8.9 ± 0.43	↓9.1 ± 0.62	+1.8	↓9.5 ± 0.72	+2.3
300	↓7.3 ± 0.62	↓8.5 ± 0.74	↓9.0 ± 0.75	↓9.2 ± 0.69	+1.9	↓9.7 ± 0.72	+2.4
1000	↓7.3 ± 0.48	↓8.3 ± 0.72	**↓7.7* ± 0.61**	**↓7.6* ± 0.74 (-22%)**	+0.5	Not reported	Not reported
	Females						
0	7.3 ± 0.18	7.8 ± 0.42	8.2 ± 0.60	8.3 ± 0.81	+1.0	8.8 ± 0.82	+1.5
30	7.3 ± 0.28	↑8.3 ± 0.47	↑8.7 ± 0.49	↑8.9 ± 0.48	+1.6	↑9.2 ± 0.55	+1.9
300	↑7.4 ± 0.32	↑8.2 ± 0.20	↑8.6 ± 0.28	↑8.7 ± 0.24	+1.3	↑9.2 ± 0.02	+1.8
1000	↓7.2 ± 0.42	↓7.0 ± 1.41	↓6.9 ± 1.21	↓6.7 ± 0.53 (-19%)	-0.5	Not reported	Not reported

* Statistically significant from controls ($p < 0.05$);

Table 6.3.2-1: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Group mean weekly body weights

Time point	Mean body weight and body weight change [kg]						
	Day 1	Week 5	Week 9	Week 11	Change week 1-11	Week 13	Change week 1-13
Dose [mg/kg bw/day]	**Males**						

Not reported: not applicable, animals sacrificed at week 11.

D. FOOD CONSUMPTION

The food consumption was not affected by the test item treatment in animals given 30 or 300 mg/kg bw/day. Reduced food consumption, varying from 25 – 75% of the amount given, was occurred on many occasions as early as Day 4 for males and Day 3 for females in animals given 1000 mg/kg bw/day. From Day 62, when tinned dog food was distributed instead of pelleted diet, all animals consumed their full daily ration.

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

The laboratory investigations of the moribund sacrificed male showed the following changes among haematological and blood biochemical parameters when compared to pre-treatment values:

- increase in leucocyte count (WBC) mainly due to an increase in the neutrophil count (N),
- increase in haemoglobin level (Hb), erythrocyte count (RBC) and packed cell volume (PCV),
- decrease in platelet count (PLAT),
- decrease in sodium and chloride levels, as well as an increase in potassium and inorganic phosphorus levels,
- increase in glucose, protein, albumin, cholesterol, triglycerides, urea and creatinine levels.

Some of the abnormalities found in the laboratory investigations (such as the increase in red blood cell parameters and in protein and albumin levels) were indicative of haemoconcentration, which was probably secondary to the dehydration caused by the diarrhoea.

When compared to both pre-dose and control values, no biologically relevant differences were noted in surviving animals of the test item groups in Weeks 7 and 11/13.

Table 6.3.2-2: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Intergroup comparison of selected haematology parameters (mean ± SD)

Parameter	Week	Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
WBC [G/L]	Pre-dose	12.91 ± 1.130	↓11.50 ± 1.524	↑13.28 ± 3.722	↑14.12 ± 1.640	11.78 ± 1.575	↓10.22 ± 2.187	↓11.24 ± 1.643	↓10.58 ± 1.771
	Week 11/13	9.47 ± 1.905	↓8.51 ± 0.135	↑13.11 ± 4.141	↑10.66 ± 1.505	10.91 ± 1.781	**↓8.57* ± 0.687**	↓8.91 ± 1.291	↓10.69 ± 2.195
N [G/L]	Pre-dose	7.99 ± 1.111	↓7.77 ± 1.694	↓7.88 ± 2.395	↑8.81 ± 1.658	7.66 ± 1.003	↓5.64 ± 1.229	↓6.78 ± 1.837	↓6.51 ± 2.384
	Week 11/13	5.72 ± 1.624	↓5.37 ± 0.325	↑8.56 ± 3.403	↑6.72 ± 1.205	7.07 ± 1.587	↓5.29 ± 0.322	↓5.21 ± 0.947	↓6.51 ± 1.956
RBC [T/L]	Pre-dose	5.59 ± 0.250	↑5.94 ± 0.344	↑6.03 ± 0.414	↑6.34 ± 0.142	6.37 ± 0.334	↑6.60 ± 0.323	↓6.36 ± 0.142	↓6.21 ± 0.396
	Week 11/13	6.94	**↓6.26* ± 0.299**	↓6.57 ± 0.511	↑7.02 ± 0.278	6.62	↑7.16 ± 0.224	↓6.60 ± 0.214	↓6.23 ± 0.585

Table 6.3.2-2: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Intergroup comparison of selected haematology parameters (mean ± SD)

Parameter	Week	Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
		± 0.169				± 0.563			
Hb [g/dL]	Pre-dose	12.7 ± 0.91	↑13.6 ± 0.75	↑13.5 ± 1.05	↑14.1 ± 0.44	14.7 ± 0.62	↑15.3 ± 0.83	14.7 ± 0.15	14.7 ± 1.0
	Week 11/13	16.5 ± 0.70	**↓14.8* ± 0.79**	**↓15.1* ± 1.14**	↓15.7 ± 0.96	15.5 ± 1.13	↑17.0 ± 0.69	15.5 ± 0.35	↓14.6 ± 1.34
PCV [L/L]	Pre-dose	0.37 ± 0.022	↑0.39 ± 0.019	↑0.39 ± 0.025	↑0.41 ± 0.015	0.42 ± 0.017	↑0.45 ± 0.022	↑0.43 ± 0.006	0.42 ± 0.031
	Week 11/13	0.50 ± 0.021	**↓0.45* ± 0.024**	↓0.46 ± 0.031	↓0.48 ± 0.021	0.47 ± 0.037	↑0.51 ± 0.018	0.47 ± 0.013	↓0.44 ± 0.042
PLAT [G/L]	Pre-dose	377 ± 32.8	↓341 ± 65.7	↓338 ± 74.9	↓359 ± 79.0	321 ± 97.4	↑381 ± 92.4	↑335 ± 4.1	↑337 ± 40.3
	Week 11/13	288 ± 21.2	↑316 ± 38.5	↑342 ± 90.2	↑373 ± 41.0	316 ± 44.8	↑327 ± 43.3	316 ± 5.7	↑423 ± 48.7

Pre-Rx: prior to first dose, Post-Rx: week 11 for 1000 mg/kg group (n=3 males, n=4 females), week 13 for other groups (n=4);
* Statistically significant from controls ($p < 0.05$).

The laboratory investigations performed before sacrifice of the moribund female dog showed the following changes among the blood biochemical parameters when compared to pre-treatment values:

- decrease in sodium, potassium, chloride and inorganic phosphorus levels,
- decrease in urea, protein and albumin levels and increase in total bilirubin level and alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities.

The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration).

Blood chemistry
When compared to control values in Week 13, the following test item related differences were noted in animals given 1000 mg/kg bw/day in Week 11 (see table below):

- higher alanine aminotransferase (ALAT) activity in 2/3 males and 1/3 females,
- lower alkaline phosphatase (ALP) activity in 3/3 females,
- lower protein and albumin levels in 3/3 females.

Other changes were not attributed to the test item treatment.

Table 6.3.2-3: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted] 2007): Group mean blood chemical values and standard deviations (SD) in Week 11/13

Dose [mg/kg bw/day]	ALAT [IU/L]	ALP [IU/L]	Total protein [g/L]	Albumin [g/L]
Males				
0 (Week 13)	31 ± 4.8	303 ± 73.7	60 ± 3.0	33 ± 1.7
30 (Week 13)	↑34 ± 5.3	↑323 ± 83.0	↓58 ± 1.8	33 ± 1.0
300 (Week 13)	↓30 ± 8.9	↑305 ± 49.5	↓58 ± 2.2	33 ± 1.3
1000 (Week 11)	↑91 ± 42.5 (+194%)	↑304 ± 53.8	60 ± 3.2	33 ± 2.1
Females				

Table 6.3.2-3: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Group mean blood chemical values and standard deviations (SD) in Week 11/13

Dose [mg/kg bw/day]	ALAT [IU/L]	ALP [IU/L]	Total protein [g/L]	Albumin [g/L]
0 (Week 13)	29 ± 6.0	388 ± 168.0	61 ± 2.1	35 ± 1.6
30 (Week 13)	↑31 ± 10.4	↓281 ± 91.5	↑62 ± 2.1	↓34 ± 1.0
300 (Week 13)	29 ± 4.1	↓332 ± 142.6	↓59 ± 2.5	35 ± 0.6
1000 (Week 11)	↑122 ± 163.9 (+321%)	↓321 ± 322.0 (-17%)	↓55 ± 5.5 (-10%)	↓30 ± 2.5 (-14%)

G. URINALYSIS

When compared to both pre-dose and control values, the following findings were noted at 1000 mg/kg bw/day in Week 11:

- decrease in mean specific gravity in 1/3 males and 3/3 females
- increase in mean urinary volume accompanied by less marked colour of urine in 3/3 females.

As these changes were only noted at the highest dose-level, they were attributed to the test item treatment.

Table 6.3.2-4: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted] 2007): Intergroup comparison of urine specific gravity values (mean ± SD)

Parameter	Week	Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
Specific gravity	Pre-dose	1043 ± 9.6	↑1045 ± 7.1	↑1044 ± 6.3	↓1038 ± 15.0	1036 ± 8.5	↑1050 ± 0.0	↑1043 ± 6.5	↑1044 ± 7.5
	Week 11/13	1045 ± 4.1	↑1048 ± 5.0	↓1043 ± 15.0	↓1028 ± 18.9	1036 ± 14.4	↑1048 ± 5.0	↑1039 ± 4.8	↓1022 ± 5.8

Pre-Rx: prior to first dose, Post-Rx: week 11 for 1000 mg/kg bw/day group (n=3), week 13 for other groups (n=4)

H. NECROPSY

Organ weights

Treatment-related, statistically significant effects were restricted to the prostate.

The RMS adds that also a statistically decreased liver weight was observed in males dosed at 1000 mg/kg bw/day (213.9 g vs 319.3 in controls, $P < 0.05$), however relative liver weight was not different between top dose males and controls (3.11 vs 3.04 in controls, weight relative to body weight).

Table 6.3.2-5: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Intergroup comparison of adrenal glands, prostate and uterus weight – absolute and relative to body weight

Organ		Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
N		4	4	4	3	4	4	4	3
Adrenal glands	Absolute [g]	1.08 ± 0.207	1.04 ± 0.187	1.27 ± 0.197	1.31 ± 0.116	1.12 ± 0.115	1.38 ± 0.440	1.17 ± 0.169	1.08 ± 0.153
	Relative [%]	0.0103 ± 0.002	0.0109 ± 0.001	0.0129 ± 0.001	**0.0176* ± 0.004**	0.0130 ± 0.002	0.0150 ± 0.004	0.0132 ± 0.002	0.0156 ± 0.002
Prostate	Absolute [g]	5.34 ± 1.58	↓4.86 ± 2.18	↓4.42 ± 1.56	**↓1.72* ± 0.308**	-	-	-	-
	Relative [%]	0.051 ± 0.016	↑0.052 ±	↓0.045 ±	↓0.023 ±	-	-	-	-

Table 6.3.2-5: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Intergroup comparison of adrenal glands, prostate and uterus weight – absolute and relative to body weight

Organ		Dose [mg/kg bw/day]							
		Males				Females			
		0	30	300	1000	0	30	300	1000
			0.025	0.012	0.002				
Uterus	Absolute [g]	-	-	-	-	9.17 ± 6.89	↓6.03 ± 4.60	↑11.88 ± 6.38	↓5.43 ± 7.62
	Relative [%]	-	-	-	-	0.109 ± 0.085	↓0.068 ± 0.056	↑0.133 ± 0.070	↓0.078 ± 0.109

* Statistically significant from controls ($p < 0.05$);
n = 3 for 1000 mg/kg bw/day groups, n=4 for all other groups

Gross pathology
Macroscopic pathological examination of the male that was killed moribund demonstrated a reddish mucosa of the colon and rectum appeared, enlarged adrenal glands and thyroids, and reduced size of the spleen and thymus.

In the high-dose female that was killed moribund, the oesophagus, jejunum and ileum presented many greyish/white areas and the colon mucosa showed reddish/purplish foci. The gall bladder was dilated with blackish deposits and the liver was yellowish, enlarged and firm. The kidneys were pale.

All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, when compared to background data, which may be seen in untreated Beagle dogs of this age, except for changes in the uterus (reduced in size) for females given 1000 mg/kg bw/day.

Histopathology
The major histopathological findings in the male dog sacrificed moribund were bilateral hyaline degeneration of the cortical tubules in the kidneys with pigment deposits, diffuse acute inflammation in the liver with pigment deposits, acute inflammation of the lamina propria of the oesophagus, bilateral hypertrophy of cortex of the adrenals, diffuse lymphoid atrophy in the spleen, acute inflammation in the lungs with alveolar spaces containing blood and increased number of adipocytes in the sternum.

The bilateral hyaline degeneration of the cortical tubules in the kidneys was considered to be test item treatment-related. However, it is not possible to determine if this lesion, which was associated with increase in urea and creatinine levels, was directly due to the test item action or the result of the dehydration caused by a severe intestinal irritation. The inflammation noted in the liver, oesophagus and lungs was considered to be test item related and was associated with change in leucocyte count. The increased number of adipocytes in the stemum seen also in the schedule killed animals was considered treatment-related. The abnormalities reported in blood electrolyte levels, glucose, triglycerides and cholesterol levels were not directly attributed to the test item treatment but were considered to be secondary to the poor clinical condition of the animal (diarrhoea, dehydration, changes in the kidneys). The modifications reported in spleen and adrenal glands were not attributed to the test item treatment, as they were non-specific changes that could be found in treated animals housed in laboratories.

At microscopic level, the major findings in the sacrificed female were bilateral vacuolation of the cortical tubules in the kidneys, macrovesicular vacuolation in the liver, diffuse hypoplasia of Langerhans islet in the pancreas, severe atrophy of cortex of the thymus, increased number of adipocytes in the sternum and uterine atrophy.

The liver histopathological modification was considered to have resulted from the test item treatment and was correlated with changes in the blood biochemical parameters (i.e. urea, protein, albumin and bilirubin levels as well as liver enzyme activities). The abnormalities reported in blood electrolyte levels were not attributed directly to the test item treatment but were related to the poor clinical condition of the animal (diarrhoea, dehydration). The uterine atrophy and increased number of adipocytes in the sternum, seen also in the schedule killed top dose animals, were considered treatment-related.

The atrophy noted in the thymus is a non-specific change that could be found in laboratory housed animals; therefore a relationship to the test treatment was excluded. The other lesions noted (i.e. in the kidneys and pancreas)

can be spontaneously observed in untreated Beagle dogs of this age and sex. Therefore, a relationship to the test treatment was considered unlikely.

No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw/day.

Treatment-related changes observed in surviving animals given 1000 mg/kg bw/day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.

These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused by the test item.

All the other microscopic findings observed in the organs of both male and female animals of the high -dose group were judged to be unrelated to treatment or normal background findings.

Table 6.3.2-6: Glyphosate Technical: 13-Week Toxicity Study by Oral Route (Capsule) in Beagle Dogs ([redacted], 2007): Summary incidence of selected histopathological findings – surviving animals

Finding	Dose [mg/kg bw/day]							
	Males				Females			
	0	30	300	1000	0	30	300	1000
Sternum: Adipocytes-increased number	0/4	0/4	0/4	3/3	0/4	0/4	0/4	3/3
Prostate: Atrophy-diffuse	0/4	0/4	0/4	2/3	-	-	-	-
Uterus: Atrophy	-	-	-	-	0/4	0/4	0/4	3/3
Adrenals: Bilateral hyperthrophy of cortex (zona fasciculata)	0/4	0/4	0/4	0/3 [a]	0/4	0/4	0/4	0/3

[a] Bilateral hyperthrophy of cortex (zona fasciculata) was only observed in the moribound male.

III. CONCLUSIONS

In the low- and mid-dose groups no treatment-related signs were noted. No haematological, blood biochemical, urinary or histopathological effects were observed. Only a slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.

At 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) or body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.

Laboratory investigations in the surviving animals demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females).

Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day. These lesions, also noted among the moribund sacrificed animals, could be related to the low body weight of these high-dose animals caused either directly or indirectly, by the test item. Further major microscopic changes in moribund sacrificed animals were found in the kidneys (bilateral vacuolation of cortical tubules, sometimes with pigment deposits), liver (diffuse macrovesicular vacuolation, acute inflammation and/or pigment deposits), oesophagus, lung, uterus (atrophy) and/or bone marrow (increased number of adipocytes). These findings were associated with numerous changes in laboratory parameters (haemoconcentration, increased urea and creatinine levels, decreased urea, protein, albumin and bilirubin levels and decreased liver enzyme activities).

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid-dose level, the No Observed Adverse Effect Level (NOAEL) was considered to be 300 mg/kg bw/day.

Assessment and conclusion by applicant:
In this study, groups of four Beagle dogs per sex received the test item, glyphosate technical, by daily administration (capsule) at dose-levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks according to OECD 409 (1998) and in compliance with GLP.

In the low- and mid-dose groups no treatment-related signs were noted. No haematological, blood biochemical, urinary or histopathological effects were observed. Only a slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.

Under the experimental conditions of the study and taking into account the slight effects on organ weights at the mid-dose level, the NOAEL is considered to be 300 mg/kg bw/day. The high dose of 1000 mg/kg bw/day was found to clearly exceed the MTD (Maximum Tolerated Dose).

Assessment and conclusion by RMS:

The RMS considers this study as acceptable and the proposed NOAEL of 300 mg/kg bw/day is agreed. At the top dose level of 1000 mg/kg bw/day, the MTD was clearly exceeded. At this dose level, clinical signs were observed (liquid/soft faeces, dehydration, vomiting) which lead to early sacrifice of two moribund animals and making termination of high dose groups after 11 weeks necessary. Further, a decreased body weight, body weight gain and food consumption was observed in both sexes, clinical chemistry and urine parameters were altered, prostate and uterus atrophy was seen and histological lesions in many organs (such as kidney liver, bone marrow) related to the moribund state of the dogs.

In males treated at 300 mg/kg bw/day, an increased absolute (+18%) and relative (+25%) adrenal weight was noted. This was also seen in males treated at 1000 mg/kg bw/day. However, as the changes in adrenal weight were not accompanied by histological changes and were also not seen in females, this finding is not considered adverse.

This conclusion is in agreement with the earlier assessment of this study in the RAR (2015): "*The study is considered acceptable and the NOAEL is agreed. At the top dose level, the MTD was clearly exceeded. It was noticed that high dose effects of glyphosate administration in this study were particularly severe, much more pronounced and rather different from what was seen in other dog studies or other species. Thus, because of the clinical signs and pathological changes, its results do not fit into the toxicity profile of glyphosate as it was established in the majority of studies. In the study by [redacted] (1990, TOX9552384) that is de scribed in detail in the original DAR (1998, ASB2010-10302), the same high dose of 1000 mg/kg bw/day was administered also in capsules causing only minor effects. There is no explanation for this apparent difference although it is known from long-term studies in rats and mice that high-dose effects of glyphosate may differ considerably. In any case, it should be taken into consideration that this dose level is by 2000 times higher than the proposed ADI.* "

B.6.3.2.16. Oral 13-week toxicity study in dogs – study 2

Data point	CA 5.3.2/021
Report author	[redacted]
Report year	1999 (study report)
Report title	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed)
Report No	1816
Document No	Not reported

Guidelines followed in study	OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Detailed clinical observations only performed monthly, not weekly. Urinalysis only performed at study termination. Several organ weights missing: epididymides, ovaries, uterus, thymus, spleen, brain, heart; several organs were not sampled: gross lesions, spinal cord, eyes with optic nerve, trachea, skin, mammary gland, prostate or other accessory sex organs. Deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point	CA 5.3.2/022
Report author	[redacted]
Report year	1999
Report title	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) - Appendix
Report No	1816
Data point	CA 5.3.2/023
Report author	[redacted]
Report year	1999
Report title	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) - List of tables
Report No	1816
Data point	CA 5.3.2/024
Report author	[redacted]
Report year	1997
Report title	Test Compound Stability in Experimental Diet (Dog feed)
Report No	1817-R.FST
Document No	Not reported
Guidelines followed in study	Not reported
Deviations from current test guideline (OECD 409, 1998)	Not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary

Three treated groups of four male and four female Beagle dogs received the test item, glyphosate technical, at dietary dose-levels of 0, 200, 2000 or 10000 ppm (corresponding to 0, 5.3, 53.5 or 252.6 mg/kg bw/day) for 90 days.

The animals were checked daily for mortality and clinical signs. Veterinary examination was carried out before grouping, at start of treatment, monthly throughout the study and at termination. Body weight was recorded

weekly. Food consumption was determined weekly. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations were performed before the beginning of the treatment period, after 45 days of exposure and at termination. Urine was analysed at termination. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.

No signs of toxicity or ophthalmological findings were observed in any dose group. Food consumption was significantly reduced in the high dose group initially (week 2) while body weights remained unaffected. The decreased food consumption is considered treatment-related and adverse as changes are >10% compared with controls.

Haematological parameters appeared in general unaffected (clotting time was increased after 45 days of exposure in both sexes, but no effects on this parameter were visible at termination; other parameters attaining statistical point significance fell within historical control). Increased GGT-levels were observed in females at the top dose at interim analysis (significant) and after 90-day treatment (not significant), which are considered adverse as there was a >50% increase compared with controls. Further, ALP was increased compared with controls at the interim blood samples in males (significant) and at both the interim and final analysis in females (both non-significant). These changes in ALP were considered adverse as the increase was >50% compared with controls. In addition, statistical significant higher levels of total bilirubin were seen at all dose levels, however, as no effects were seen on the liver, only the increased levels at the top dose were considered adverse as these were accompanied by increased GGT- and ALP levels. No effects on urine parameters, organ weights or organ histopathology were observed.

The RMS proposes a NOAEL of 2000 ppm (corresponding to 54.2 and 52.8 mg/kg bw/day in males and females, respectively) based on decreased food consumption and increased GGT and ALP-levels at the top dose of 10000 ppm.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Crystalline solid
Lot/Batch#:	01.12.1997 & 01.06.1997
Purity:	>95%
Stability of test compound:	Expiry dates: 2000-06-01 and 2000-12-01

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Dogs
Strain:	Beagle
Source:	[redacted] (in-house breed)
Age:	6 – 8 months
Sex:	Male and female
Weight at dosing:	♂ 10.0 – 12.2 kg; ♀ 8.8 – 11.0 kg
Acclimation period:	6 days
Diet/Food:	Nutripet Pet meal (Tetragon Chemie Pvt.Ltd., Bangalore, India), was offered daily for one hour, *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*

Housing:	Individual housing in floor pens.	
Environmental conditions:	Temperature:	23 – 29 °C
	Humidity:	40 – 70%
	Air changes:	Not reported
	Natural daylight plus fluorescent light from 9 am to 5 pm	

B: Study design and methods

In life dates: 1998-03-18 to 1998-06-26

Animal assignment and treatment:
In a 90 day feeding study groups of four Beagle dogs per sex received daily doses of 0, 200, 2000 or 10000 ppm glyphosate technical in the diet (equivalent to 5.3, 53.5 or 252.6 mg/kg bw/day).

Test diets were prepared prior to start of treatment and then twice during the three month study period by mixing a known amount of the test substance with a small amount of basal diet and blending. This pre-mix was then added to a larger amount of basal diet and blended for 20 minutes. The feed was fortified with test compound at weekly intervals.

The stability of the test compound was examined in an additional study (No. 1817-R.FST). The homogeneity of the test material in diet was determined at start of the study. Three samples from the food fortified with the test compound were taken and analysed.

Mortality
Each animal was checked for mortality or signs of morbidity daily during the treatment period.

Clinical observations
Each animal was daily checked for signs of toxicity. A more detailed veterinary investigation was performed before start of exposure, monthly throughout the study and before termination.

Body weight
The body weight of each animal was recorded before allocation and start of treatment, weekly throughout the study and before termination.

Food consumption
The quantity of food consumed was recorded for each animal on a weekly basis. Food was offered in a dedicated bowl for a period of 1 hour after which any remaining or spilled food was removed.

Ophthalmoscopic examination
Ophthalmological examinations were performed on all the animals before the beginning and at the end of the treatment period.

Haematology and clinical chemistry
Haematological, blood chemical and urinalytical investigations were performed on all animals from each test and control group before the beginning of the treatment period, after 45 days of exposure and at termination from animals fasted since the last feeding (approximately 23 hours).

The following parameters were determined: Erythrocytes (RBC), haemoglobin (HB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), leucocytes (WBC), differential white cell count (Neutrophils (Neut), lymphocytes (Lymp), eosinophils (Eosi), monocytes (Mono), reticulocytes (Retic)), clotting time, glucose, blood urea nitrogen, total protein, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatinine, total bilirubin, albumin, calcium, inorganic phosphorous, total cholesterol, triglycerides, chloride, sodium and potassium.

Urinalysis
Urine was collected from all animals at termination during autopsy. Urinalysis was performed for control and high-dose group animals. The following parameters were determined: Specific gravity, pH, leucocytes, nitrite, proteins, glucose, ketones, blood, bilirubin and urobilinogen.

Sacrifice and pathology
On completion of the treatment period, after an overnight fasting, all surviving animals were subjected to a gross pathological examination. The moribund animals were sacrificed in the same way. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, kidneys, liver (with gall bladder), testes and thyroids with parathyroids.

Tissue samples were taken from the following organs and preserved in buffered formalin: Adrenals, aorta, bone & bone marrow (sternum), brain (medulla, pons, cerebellum and cerebrum), caecum, colon, duodenum, gall bladder, gonads, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mesenteric), oesophagus, pancreas, pituitary gland, rectum, salivary glands, sciatic nerve, spleen, stomach, thymus, thyroids with parathyroids, urinary bladder, uterus. These tissues (plus parathyroids) were microscopically investigated for all animals of the control and high dose group. Further histopathology in other dose groups were carried out on all gross lesions.

Statistics
Body weights, net body weight gain, food intake, laboratory investigations (haematology and clinical chemistry values of days 0, 45 and 90), organ weights data and organ weight ratios 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. Following ANOVA, when F was found to be significant, Dunnett's pair wise comparison of means of treated groups with control mean was done individually. Following a significant difference of a test group with the control group, the dose-response correlation was estimated including the control and all treated groups and tested by t-test. All analyses and comparisons are evaluated at 5 % probability level.

II. RESULTS AND DISCUSSION

ACHIEVED DOSAGES AND ANALYSIS OF THE FORMULATED DIETS
The prepared experimental food was sampled for assay of test compound concentration analysis at start of treatment (1 batch), at 7th week and the last batch of mixing before termination. The mean active ingredient concentrations determined for the above batches of food mixed is 0.0 for the control group, 188.9 ±2.83 for the 200 ppm dose group, 1941.9 ± 61.99 for the 2000 ppm dose group and 9562.6 ± 415.80 for the 10000 ppm dose groups. The results indicate that the test compound concentration in the experimental food prepared was within the permissible limit of (± 10%) of variation.

Test compound homogeneity in dog food was determined at the start of treatment (in one batch). The mean glyphosate concentration determined for the four batches of food mixed 0.0 for the control group, 190.8 ± 4.30 for the 200 ppm dose group, 1888.9 ± 45.77 for the 2000 ppm dose groups and 9808.7 ± 236.48 for the 10000 ppm dose group. The results show that the experimental food prepared is homogeneous.

Glyphosate Technical was found to be stable in the dog feed up to 30 days of storage at the ambient temperature with a slight but acceptable reduction of 9.6% and 5.0% in the test compound concentration at 200 and 10000 ppm, respectively, at the end of this period.

A. MORTALITY
All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed.

C. BODY WEIGHT
Body weights and body weight gains remained statistically unaffected by treatment in both males and females. No weight loss was observed.

Table 6.3.26-1: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) [redacted] 1999): Group mean weekly body weights

	Body weight [kg]						
Week	**Initial**	**1**	**2**	**3**	**4**	**5**	**6**
Test item [ppm]	**Males**						
0	11.1 ± 0.8	11.7 ± 0.6	12.2 ± 0.5	12.3 ± 0.6	12.5 ± 0.6	12.7 ± 0.5	12.9 ± 0.7
200	↑11.2 ± 0.7	↓11.6 ± 0.5	↓12.1 ± 0.4	12.3 ± 0.6	↑12.8 ± 0.6	12.7 ± 0.5	↑13.2 ± 0.7
2000	11.1 ± 0.7	11.7 ± 0.4	↓12.1 ± 0.6	↓12.0 ± 0.6	↑12.6 ± 0.6	12.7 ± 0.6	12.9 ± 0.6
10000	11.1 ± 0.9	↓11.4 ± 0.9	↓11.2 ± 0.8	↓11.5 ± 0.9	↓12.4 ± 0.6	↑12.4 ± 0.8	↓12.7 ± 0.5
	Females						
0	10.1 ± 1.0	10.4 ± 1.2	10.9 ± 0.9	10.7 ± 1.3	11.0 ± 1.3	11.0 ± 1.3	11.2 ± 1.5
200	↑10.2 ± 0.9	↑10.7 ± 1.2	↑11.1 ± 0.9	↑10.9 ± 1.2	↑11.3 ± 1.4	↑11.4 ± 1.3	↑11.5 ± 1.4
2000	↓9.8 ± 0.8	↓10.2 ± 0.7	↓10.3 ± 0.9	↓10.2 ± 0.4	↓10.8 ± 0.7	↓10.9 ± 0.7	↓11.0 ± 1.0
10000	↓10.0 ± 0.5	↓9.9 ± 0.6	↓10.0 ± 0.9	↓10.1 ± 0.9	↓10.6 ± 0.8	↓10.6 ± 0.8	↓10.7 ± 1.0
	Body weight [kg]						
Week	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Test item [ppm]	**Males**						
0	13.2 ± 0.7	13.3 ± 0.5	13.4 ± 0.4	13.5 ± 0.5	13.6 ± 0.4	13.7 ± 0.5	13.8 ± 0.3
200	↑13.5 ± 0.6	↑13.5 ± 0.6	↑13.5 ± 0.8	↑13.6 ± 0.8	↑13.7 ± 0.8	↓13.6 ± 0.9	↓13.5 ± 0.9
2000	13.2 ± 0.7	↑13.4 ± 0.7	13.4 ± 0.8	↓13.3 ± 0.9	↓13.3 ± 0.9	↓13.2 ± 1.0	↓13.2 ± 1.1
10000	↓12.8 ± 0.9	↓13.0 ± 0.9	↓13.3 ± 0.8	↓13.3 ± 0.8	↓13.5 ± 0.9	↓13.3 ± 0.9	↓13.6 ± 1.0
	Females						
0	11.4 ± 1.6	11.7 ± 1.4	11.9 ± 1.4	11.7 ± 1.6	12.0 ± 1.3	11.7 ± 1.3	11.8 ± 1.3
200	↑11.7 ± 1.4	↑11.8 ± 1.4	↓11.5 ± 1.6	↓11.6 ± 1.5	↓11.6 ± 1.6	↓11.6 ± 1.6	↓11.4 ± 1.7
2000	↓11.2 ± 0.9	↓11.4 ± 1.0	↓11.3 ± 1.2	↓11.4 ± 1.2	↓11.5 ± 1.2	↓11.6 ± 1.2	↓11.5 ± 1.5
10000	↓10.9 ± 1.2	↓11.2 ± 1.2	↓11.3 ± 1.1	↓11.3 ± 1.1	↓11.3 ± 1.1	↓11.3 ± 1.0	↓11.2 ± 1.3

C. FOOD CONSUMPTION

The food intake of the high dose group (10000 ppm) was significantly lower during the second week of treatment only. Except for this finding the food consumption of all the treatment groups was comparable to the control group during the study period.

Table 6.3.26-2: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) [redacted] 1999): Average weekly food intake

	Food consumption [g/animal/day]						
Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Test item [ppm]	**Males**						
0	254 ± 84.18	336 ± 34.76	342 ± 38.72	336 ± 36.74	320 ± 48.33	346 ± 13.88	345 ± 32.11
200	↑287 ± 11.12	↑354 ± 22.38	↑366 ± 42.98	↑376 ± 33.24	↑326 ± 41.72	346 ± 19.30	↑350 ± 22.46
2000	↑305 ± 34.04	↑373 ± 32.62	↑406 ± 29.60	↑347 ± 52.39	↑334 ± 27.28	↑363 ± 18.51	↑358 ± 41.16
10000	↑262 ± 14.66	**↓177* ± 21.05 (-47%)**	↑380 ± 69.48	↑368 ± 40.18	↑342 ± 14.68	↓332 ± 17.02	↓330 ± 23.85
Test item [ppm]	**Females**						
0	247 ± 48.56	263 ± 64.46	278 ± 64.82	302 ± 52.58	295 ± 48.42	298 ± 41.60	283 ± 43.19

Table 6.3.26-2: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) [redacted] 1999): Average weekly food intake

	Food consumption [g/animal/day]						
Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Test item [ppm]	**Males**						
200	↑285 ± 41.19	↑332 ± 44.57	↑324 ± 11.87	↑352 ± 37.92	↑323 ± 47.21	↑321 ± 46.27	↑292 ± 41.66
2000	↓212 ± 56.44	↑306 ± 35.34	↑338 ± 39.11	↑309 ± 40.42	↑298 ± 35.71	↓286 ± 40.52	↑290 ± 25.84
10000	↓212 ± 56.85	**↓166* ± 49.84 (-37%)**	↑348 ± 38.54	↑327 ± 12.23	↑303 ± 32.63	↓261 ± 73.55	↑288 ± 51.41

	Food consumption [g/animal/day]					
Week	**8**	**9**	**10**	**11**	**12**	**13**
Test item [ppm]	**Males**					
0	325 ± 32.85	312 ± 36.42	332 ± 30.75	356 ± 34.71	368 ± 39.65	369 ± 39.22
200	↑342 ± 9.63	↑325 ± 27.23	↑343 ± 23.34	↓318 ± 31.48	↓347 ± 37.37	↓312 ± 38.62
2000	↑366 ± 27.74	↑339 ± 35.81	↓321 ± 59.31	↓330 ± 43.36	↓329 ± 50.93	↓339 ± 41.15
10000	↓324 ± 1.50	↑328 ± 14.35	↓303 ± 20.89	↓341 ± 8.66	↓333 ± 29.42	↓330 ± 20.32
Test item [ppm]	**Females**					
0	297 ± 42.76	275 ± 40.08	290 ± 32.70	295 ± 29.64	292 ± 24.20	285 ± 30.58
200	↓294 ± 68.51	↑294 ± 40.30	↑325 ± 19.41	↑317 ± 54.56	↓290 ± 83.86	↓271 ± 68.36
2000	↑298 ± 26.68	↑284 ± 39.81	↓278 ± 22.77	↓285 ± 20.64	↑303 ± 33.72	↓283 ± 36.84
10000	↓294 ± 40.57	↑298 ± 42.13	↓262 ± 31.61	↓268 ± 35.84	↓262 ± 40.44	↓256 ± 27.40

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

The calculated mean daily test substance intake is summarised in Table 6.3.2.16-3.

Table 6.3.2.16-3: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) [redacted] 1999): Group mean compound intake levels

Dose group	Dietary concentration [ppm]	Mean daily test substance intake [mg/kg bw/day]*		
		Males	Females	Combined
1 (control)	0	0.0	0.0	0.0
2 (low)	200	5.2	5.4	5.3
3 (mid)	2000	54.2	52.8	53.5
4 (high)	10000	252.4	252.7	252.6

* Based on actual food intake and body weight data

D. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the beginning and at the end of the treatment period.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item. The RMS has added data on alkaline phosphatase (ALP) in table 6.3.2.17-4, which showed an increase compared with controls at the interim blood samples in males (significant) and at both the interim and final analysis in females (non-significant).

Table 6.3.26-4: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) ([redacted] 1999): Summary of results for clotting time, GGT-activity and alkaline phosphatase.

	Males				Females			
[ppm]	**0**	**200**	**2000**	**10000**	**0**	**200**	**2000**	**10000**
	Clotting time [s]							
Pre-exposure bleed	145 ± 32.47	↑150 ± 22.37	↑147 ± 12.69	↓144 ± 37.47	154 ± 28.62	↑162 ± 17.31	↓149 ± 17.17	↓131 ± 24.21
45 day interim bleed	131 ± 3.77	**↑153* ± 4.76**	**↑172* ± 6.61**	**↑183*$ ± 2.94**	141 ± 6.68	**↑161* ± 1.50**	**↑173* ± 8.66**	**↑182*$ ± 2.36**
90 d final bleed	134 ± 10.85	134 ± 18.84	↑136 ± 12.40	↑139 ± 4.79	142 ± 7.89	142 ± 10.08	↓134 ± 11.90	↓138 ± 21.92
	GGT [U/L]							
Pre-exposure bleed	9 ± 2.5	↑10 ± 2.16	↓8 ± 2.31	↓7 ± 0.82	9 ± 3.51	↓7 ± 2.58	↓7 ± 1.89	↑11 ± 5.56
45 day interim bleed	13 ± 3.56	13 ± 1.91	↑16 ± 1.73	**↑19*$ ± 2.22 (+171%)**	14 ± 1.71	14 ± 3.74	14 ± 2.94	**↑21* ± 2.87 (+91%)**
90 d final bleed	11 ± 7.27	↑12 ± 2.00	↑16 ± 4.50	↑18 ± 1.50	17 ± 6.06	↓16 ± 5.56	↓16 ± 6.99	↑29 ± 7.89
	ALP (U/L)							
Pre-exposure bleed	151 ± 37.61	146 ± 21.09	193 ± 57.43	189 ± 62.43	155 ± 62.75	135 ± 43.89	155 ± 27.21	185 ± 53.82
45 day interim bleed	259 ± 114.6	232 ± 32.15	384 ± 102.6	**432* ± 69.70 (+129%)**	309 ± 147.5	264 ± 104.2	318 ± 74.87	427 ± 135.8
90 d final bleed	313 ± 164.2	228 ± 58.82	317 ± 115.9	371 ± 81.36	305 ± 180.4	233 ± 106.5	268 ± 105.5	465 ± 75.27

* Statistically significant from controls ($p \leq 0.05$);
$ Significant dose correlation

Total bilirubin seemed affected; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

Table 6.3.26-5: Subchronic (90 Day) Oral Toxicity Study with Glyphosate Technical in Beagle Dogs & Test compound stability in experimental diet (dog feed) [redacted] 1999): Summary of results for total bilirubin

	Males				Females			
[ppm]	**0**	**200**	**2000**	**10000**	**0**	**200**	**2000**	**10000**
	Total bilirubin [µmol/L]							
Pre-exposure bleed	3.71 ± 0.43	↑3.99 ± 0.39	3.71 ± 0.57	↓3.14 ± 0.24	3.67 ± 0.35	↓3.51 ± 0.62	↑3.96 ± 0.80	↑4.02 ± 0.36
45 day interim bleed	5.25 ± 0.75	↓5.10 ± 0.70	↑5.93 ± 0.45	↑5.97 ± 0.26	5.22 ± 0.30	↑5.23 ± 0.38	**↑6.49* ± 0.47**	**↑6.54*$ ± 0.34**
90 d final bleed	4.21 ± 0.90	**↑5.65* ± 0.54**	**↑5.95* ± 0.73**	**↑6.21*$ ± 0.54 (+98%)**	4.00 ±0.52	**↑6.57* ± 0.29**	**↑7.08* ± 0.35**	**↑7.18*$ ± 0.75 (+79%)**

* Statistically significant from controls ($p \leq 0.05$);
$ Significant dose correlation

F. URINALYSIS
All parameters were in the normal range and comparable between control and treated animals.

G. NECROPSY
Organ weights
No treatment-related effects were observed.

Necropsy
No treatment-related effects were observed.

Histopathology
There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.

III. CONCLUSIONS

No signs of toxicity or ophthalmological findings were observed in any dose group. Food consumption was significantly reduced in the high dose group initially (week 2) while body weights remained unaffected.
Haematological parameters appeared in general unaffected (clotting time was increased after 45 days of exposure in both sexes, but no effects on this parameter were visible at termination; other parameters attaining statistical point significance fell within historical control). Slight increases on total bilirubin and gamma-glutamyl-transferase were observed in the high dose group. No effects on urine parameters, organ weights or organ histopathology were observed.

In absence of any histopathological correlate, the inconsistent effects described in haematology and clinical chemistry were considered incidental and of no toxicological significance. The No Observed Adverse Effect Level (NOAEL) was considered to be 10000 ppm.

Assessment and conclusion by applicant:
In this study, three treated groups of four male and four female Beagle dogs received the test item, glyphosate technical, at dietary dose levels of 0, 200, 2000 or 10000 ppm (corresponding to 0, 5.3, 53.5 or 252.6 mg/kg bw/day) for 90 days according to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Oral administration of glyphosate technical via feed to Beagle dogs at concentrations of 200, 2000 or 10000 ppm had no adverse effect on general health, growth, haematological, clinical chemistry or urinalysis parameters, organ weights, gross or histopathological changes. The NOAEL is considered to be 10000 ppm, corresponding to 252.6 mg/kg bw/day.

Assessment and conclusion by RMS:
The RMS disagrees with the notifier on the NOAEL of the study and proposes a lower NOAEL of 2000 ppm (corresponding to 54.2 and 52.8 mg/kg bw/day in males and females, respectively). This is based on a decreased food consumption in both sexes in the second week of treatment and on increased GGT-levels in females at the top dose at interim analysis (significant) and after 90-day treatment (not significant), which are >50% increased compared with controls.
Further, ALP was increased compared with controls at the interim blood samples in males (significant) and at both the interim and final analysis in females (both non-significant). These changes in ALP were considered adverse as the increase was >50% compared with controls. In addition, statistical significant higher levels of total bilirubin were seen at all dose levels, however, as no effects were seen on the liver, only the increased levels at the top dose were considered adverse as these were accompanied by increased GGT - and ALP levels.

In the previous assessment in the RAR (2015), the NOAEL proposed by the notifier was agreed and the following was concluded by RMS DE: "*The study is considered acceptable. It is agreed to consider the highest dose the NOAEL because the minor effects were indeed not adverse. The lower body weight gain at the beginning of treatment is very probably a result of impaired food consumption. Lower food intake might be due to a palatability problem or might simply result from the need of the animals to adapt to a diet with a new*

and perhaps strange taste. The higher bilirubin levels might be due to treatment but were not accompanied by any pathological change.
It was noted that the highest dose choosen, as compared to other studies with dietary administration to dogs, was rather low."

B.6.3.2.17. Oral 13-week toxicity study in dogs – study 3

Data point	CA 5.3.2/025
Report author	[redacted]
Report year	1996 (Study report)
Report title	First Revision to Glyphosate Acid: 90-Day Oral Toxicity Study in Dogs
Report No	[redacted] P/1802
Document No	Not reported
Guidelines followed in study	OECD 409 (1981); US EPA Subdivision F 82-1(b)
Deviations from current test guideline (OECD 409, 1998)	Heart, thymus, spleen and uterus were not weighed; microscopic examination of spinal cord was performed only at lumbar level. Deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point	CA 5.3.2/026
Report author	[redacted]
Report year	1996
Report title	First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs - Appendix
Report No	[redacted] P/1802

Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary
In a repeated-dose toxicity study glyphosate acid was administered to groups of four male and four female Beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm (equivalent to 0, 68, 323 or 1680 mg/kg bw/day for males and 0, 68, 334 or 1750 mg/kg bw/day for females) glyphosate acid in the diet for a period of at least 90 days. The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined microscopically.

Glyphosate acid was palatable to dogs in dietary concentrations up to and including the limit dose of 50000 ppm in the diet.

Toxic effects were confined to dogs given 50000 ppm glyphosate acid, these being small reductions of body weight gain. Males also had slightly reduced plasma protein and calcium concentrations.

Kidney weights of males given 10000 and 50000 ppm glyphosate acid were increased. Plasma alkaline phosphatase activity of females given 50000 ppm glyphosate acid was slightly increased. These effects were not accompanied by a histopathological lesion and are considered to be of no toxicological significance.

There were no haematological, clinical or pathological changes associated with glyphosate acid treatment. The toxicological no observed adverse effect level of glyphosate acid given in the diet to dogs for 90 days was 10000 ppm, with only minimal effects at 50000 ppm.

An absolute no effect level was 2000 ppm glyphosate acid.

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm.

The RMS agrees with the NOAEL of 10000 ppm as proposed by the notifier. This dose level is equivalent to 323 mg/kg bw/day for males and 334 mg/kg bw/day for females. The increased absolute kidney weights at the mid and high-dose in males are not considered adverse as no clear dose-response was shown. No data is provided a relative kidney weights. At the mid and high dose, absolute liver weight was also increased, however, as these changes do not exceed 20% compared with controls this is not considered adverse. At the top dose of 50000 ppm, also an adverse decrease in body weight gain in males and females and a decreased in plasma calcium levels in males was seen.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate acid

Description:	Technical, white solid (passed through a 75 µm mesh)
Lot/Batch number:	D4490/1, P18
Purity:	99.1 % w/w a.s.
CAS#:	Not reported
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Glyphosate acid was administered in diet

3. Test animals:

Species	Dog
Strain	Beagle
Age/weight at dosing	22 – 26 weeks
Source	[redacted]
Housing	Individually in indoor pens, with a floor area of 345 × 115 cm. Each pen consisted of an exercise area and separate sleeping quarters with a heated floor
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Witham, Essex, UK), *ad libitum*
Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 19 – 22 °C Humidity: Not reported Air changes: Approximately 12 changes / hour Photoperiod: 11 hours light / 13 hours dark

B: Study design and methods

In-life dates: 12 August 1986 19 November 1986

Animal assignment
The study consisted of one control and three treatment groups each containing 4 male and 4 female dogs. The randomisation procedure employed ensured the even distribution of animals across replicates (randomised blocks) and treatment groups, by body weight, placing litter mates in different treatment groups. The sexes were randomised separately.

Male dogs received 400 g and females 350 g of the appropriate diet, in the morning between 9 am and 12 am each day. During the pre-study period, the food was removed 2 – 5 hours after presentation in an attempt to ensure that the dogs ate the diet rapidly. Several batches of test diets were prepared so that no one batch was fed for longer than 5 weeks.

The clinical condition and body weights of the dogs were monitored during the study, as was their biochemical and haematological status. At the end of the study the dogs were subjected to an examination *post mortem*. The major organs were fixed, processed and examined.

Diet preparation and analysis
All experimental diets were based on expanded, ground Laboratory Diet A.

The glyphosate acid concentration was determined for each occasion diet was mixed. The homogeneity of diets containing glyphosate acid was established by analysis of aliquots of diet taken from each mix of the low and high dose diet on the first occasion on which diets were prepared. The stability of the low and high dose diets was determined over a 39 day period on one mix from the first occasion on which diets were prepared.

Concentration analysis results: The achieved dietary concentrations of glyphosate acid were all within ± 9 % of the target concentrations.

Homogeneity results: The homogeneity was considered to be satisfactory with all the mean values from the analysis at the different sampling points being within 6 % of the overall mean.

Stability results: Over a period of 39 days, no significant change was seen in the chemical stability at 2000 and 50000 ppm glyphosate acid.

Observations:
A detailed clinical examination, which included cardiac and pulmonary auscultation, was made on all dogs pre-experimentally and in week 13. In the treatment period, the dogs were observed at least twice during the working day for gross clinical and behavioural abnormalities.

A daily record of faecal consistency was made during the pre-experimental and dosing periods.

Body weight
All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals, until termination.

Food consumption
Food residues were recorded daily and were then discarded. These measurements were made usually 4 hours (between 2 – 5 hours) after presentation of the diet during the pre-experimental period and approximately 24 hours after presentation of the diet during the dosing period.

Ophthalmoscopic examination
The eyes of all dogs were examined by indirect ophthalmoscopy pre-experimentally and in week 13.

Haematology and clinical chemistry
Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelet count, total white cell count, differential white cell count, blood cell morphology, kaolin-cephalin time and prothrombin time.

Bone marrow smears were taken from a femur of all dogs at necropsy, air dried, fixed in absolute methanol and stored but not examined.

Clinical chemistry
Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters assessed in plasma: Urea, glucose, albumin, total protein, cholesterol, triglycerides, creatine kinase activity, alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma-glutamyl transferase activity, calcium, sodium and potassium.

Urinalysis
Urine was collected by catheterisation from all dogs, once pre-experimentally and in week 13. Microscopic examination of the centrifuged deposits, from all dogs, was made pre-experimentally and in week 13 on the samples taken for biochemical analysis. The following parameters were determined: Urobilinogen, specific gravity, pH, bilirubin, protein, ketones, glucose and blood.

Investigations *post mortem*
Macroscopic examination
At the end of the 90 day dosing period, all animals were killed and examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights
From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed: Adrenal glands, brain, epididymides, kidneys, ovaries, liver, testes and thyroid glands (with parathyroids).

The left and right components of paired organs were weighed separately.

Tissue submission
The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative: Gross lesions including masses, adrenal gland, aorta, brain, bone and bone marrow (rib), caecum, colon, duodenum, gall bladder, epididymis, eyes, femur (including stifle joint), heart, ileum, jejunum, kidney, liver, lung, lymph node – prescapular, lymph node – mesenteric, mammary gland (females only), oesophagus, ovary, pancreas, pituitary gland, prostate gland, rectum, salivary gland, spinal cord (lumbar), skin, spleen, sternum, stomach, testis, thymus, thyroid/parathyroid gland, trachea, urinary bladder, uterus, voluntary muscle, cervix and nerve – sciatic.

Microscopic examination
All processed tissues were examined by light microscopy.

Statistics
Body weight gains from the start of the study to each week and final body weights were considered by analysis of variance, separately for males and females.

Haematology, blood and urine biochemistry data were considered, at each sampling time after the start of the study, by analysis of co-variance on pre-experimental values. Male and female data were analysed together and the results examined to determine whether differences between control and treated groups were consistent between sexes.

Organ weights at termination were considered by analysis of variance and analysis of co-variance on the last measured body weight, separately for males and females. Left and right components of paired organs were considered separately and combined to investigate for any differential effects.

All analyses allowed for the replicate design of the study and were carried out using SAS (1982). Unbiased estimates of the treatment group means were provided by least square means (LSMEANS option in SAS). Each treatment group was compared to the control group mean using a two-sided Student's t-test, based on the error mean square from the appropriate analysis. Where male and female data were analysed together, these comparisons were made separately.

All data were checked for atypical values and where such values were detected the analyses were repeated omitting these values to determine their influence on the conclusions.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

The clinical observations noted were of a minor nature, often seen in studies of this duration using this strain of dog and are considered to be unrelated to treatment with glyphosate acid.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight gain of males given 50000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant.

Females given 50000 ppm glyphosate acid showed slightly reduced body weight gains throughout the study and these were occasionally statistically significantly different from the controls.

There was no effect on growth in dogs given 2000 or 10000 ppm glyphosate acid.

Table 6.3.2-1: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of body weight and body weight gain [g] (selected time points)

Week	Dietary Concentration of glyphosate acid [ppm]							
	Males				Females			
	0	2000	10000	50000	0	2000	10000	50000
Initial weight	10.97	10.60	11.00	10.90	9.70	9.40	9.47	9.47
4	1.00	1.13	1.07	0.65 (-35%)	0.64	0.75	0.85	**0.38* (-41%)**
9	2.07	1.92	2.07	1.65 (-20%)	1.31	1.42	1.52	**0.97* (-26%)**
13	2.47	2.93	2.70	2.03 (-18%)	1.60	1.72	1.92	1.47 (-8%)
Final weight	13.03	13.00	13.37	12.50	11.31	11.13	11.40	10.95

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided)

D. FOOD CONSUMPTION AND UTILISATION

All dogs ate all the diet presented during the dosing period. The dose received (in mg glyphosate acid/kg bw/day) was similar for both males and females. During the study, there was the expected decrease in the dose received, due to the increasing weight of the dogs.

One dog fed 10000 ppm glyphosate acid was given cubed diet for two days in week 5 to prevent it scooping up powdered diet and thereby allowing healing to a wound in its front paw. No glyphosate acid was received by this dog on these two days.

Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg bw/day. Mean values are shown below:

Table 6.3.2-2: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] 1996): Mean Dose Received [mg/kg bw/day]

Glyphosate acid [ppm]	2000	10000	50000
Males	68	323	1680
Females	68	334	1750

E. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmological findings.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry

Male dogs fed 50000 ppm glyphosate acid showed slightly reduced plasma albumin and total protein concentrations, possibly representing the start of the expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Plasma calcium levels were also minimally reduced in these animals, possibly a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid.

Female dogs given 50000 ppm glyphosate acid had slightly elevated plasma alkaline phosphatase activities throughout the study.

There were no treatment-related changes in dogs fed 2000 or 10000 ppm glyphosate acid. There were other isolated instances where results were statistically significantly different from control, but these were considered to be unrelated to treatment.

Table 6.3.2-3: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of clinical chemistry – selected parameters and time points

Parameter	Week	Dose Level of glyphosate acid [ppm]							
		Males				Females			
		0	2000	10000	50000	0	2000	10000	50000
Albumin [g/100 mL]	4	3.70	3.70	↑3.73	↓**3.43***	3.76	↓3.65	↑3.89	↓**3.51***
	8	3.77	↓3.74	↓3.69	↓**3.53***	3.72	↓3.71	↑3.92	↓3.63
	13	3.92	↑3.97	↓3.77	↓**3.66***	3.84	↓3.70	↑3.94	↓3.78
Total protein [g/100 mL]	4	5.57	↓5.42	↓5.34	↓**5.14****	5.36	↑5.40	↑5.42	↓5.22
	8	5.44	↑5.49	↓5.32	↓**5.22***	5.32	↓5.30	↑**5.52***	↓5.19
	13	5.60	↑5.70	↓5.45	↓5.38	5.39	↓5.34	↑**5.65***	↓5.30
Calcium [mg/100 mL]	4	11.2	11.2	↓11.1	↓**10.5**** **(-6%)**	10.9	↑11.1	↑11.2	↓10.7
	8	11.2	↓11.1	↓**10.9***	↓**10.8**** **(-4%)**	10.9	↑11.0	↑**11.2***	10.9
	13	10.7	↓10.5	↑10.8	↓**10.0**** **(-7%)**	10.4	↓10.3	↑10.6	10.4
	4	182	↑190	↑188	↑193	176	↑181	↑182	↑**220****
	8	155	↑168	↑164	↑177	152	↑155	↑155	↑**181***

Table 6.3.2-3: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of clinical chemistry – selected parameters and time points

Parameter	Week	Dose Level of glyphosate acid [ppm]							
		Males				Females			
		0	2000	10000	50000	0	2000	10000	50000
Plasma alkaline phosphatase [mU/mL]	13	149	↑165	↑160	↑161	140	↑143	↑145	**↑166***

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$; Student's t-test, 2-sided)

G. URINALYSIS

There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.

F. SACRIFICE AND PATHOLOGY

Organ weights

Kidney weights of males given 10000 or 50000 ppm glyphosate acid were slightly increased above control values. There was also a small increase in liver weight at these dose levels, but in male dogs only.

Thyroid weights, adjusted for body weight, of females given 2000 or 10000 ppm glyphosate acid were statistically significantly reduced from control values. In the absence of any dose response relationship across all groups, this is considered not to be of toxicological significance.

Table 6.3.2-4: First Revision to Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of selected organ weights [g] in dogs (absolute and adjusted (g/13 kg body weight))

Organ	Dose Level of glyphosate acid [ppm]							
	Males				Females			
	0	2000	10000	50000	0	2000	10000	50000
Kidneys – absolute (g)	56.1	57.7	**62.4***	**62.0***	52.6	50.6	54.4	55.6
Kidneys – adjusted (g/13 kg body weight)	55.7	58.2	**61.6***	**62.7***	53.6	50.4	53.0	56.2
Liver – absolute (g)	385	409	**427****	**436****	358	358	373	359
Liver – adjusted (g/13 kg body weight)	385	409	**427***	**436****	362	357	367	362
Thyroid – absolute (g)	0.902	0.952	1.011	0.871	0.926	0.815	0.767	0.847
Thyroid – adjusted (g/13 kg body weight)	0.893	0.966	0.989	0.890	0.949	0.811*	**0.735****	0.860

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$; Student's t-test, 2-sided)

Macroscopic findings

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

Microscopic findings
There was no microscopic pathology attributable to the administration of glyphosate acid.

Incidental findings included minor granulomatous/inflammatory lesions in lung, alimentary tract and lymph node associated with ascarid migration. Imperfect spermatogenesis and minimal secretory activity of the prostate were observed in several sexually immature males. Minimal cystitis manifest as infiltration of the mucosa by inflammatory cells and small haemorrhages were found in several animals and were consistent with a subclinical bacterial infection of the lower urinary tract.

III. CONCLUSIONS

Glyphosate acid was palatable to dogs in dietary concentrations up to and including the limit dose of 50000 ppm in the diet.

Toxic effects were confined to dogs given 50000 ppm glyphosate acid, these being small reductions of body weight gain. Males also had slightly reduced plasma protein and calcium concentrations.

Liver and kidney weights of males given 10000 and 50000 ppm glyphosate acid were increased. Plasma alkaline phosphatase activity of females given 50000 ppm glyphosate acid was slightly increased. These effects were not accompanied by a histopathological lesion and are considered to be of no toxicological significance.

There were no haematological, clinical or pathological changes associated with glyphosate acid treatment. The toxicological no observed adverse effect level of glyphosate acid given in the diet to dogs for 90 days was 10000 ppm, with only minimal effects at 50000 ppm.

An absolute no effect level was 2000 ppm glyphosate acid.

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no adverse effect level for glyphosate acid from this study was 10000 ppm in the diet (equivalent to a dose of more than 300 mg glyphosate acid/kg bw/day).

Assessment and conclusion by applicant:
In this sub-chronic toxicity study glyphosate acid was administered to groups of four male and four female Beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm (equivalent to 0, 68, 323 or 1680 mg/kg bw/day for males and 0, 68, 334 or 1750 mg/kg bw/day for females) glyphosate acid in the diet for a period of at least 90 days according to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Minimal toxicity including minimally reduced body weight gain was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. Reduced plasma albumin and total protein concentrations in male dogs fed 50000 ppm glyphosate acid may represent the start of the expected effect of feeding an inert substance at a sufficiently high level to reduce the intake of nutrients. Minimal reduction of plasma calcium may be a result of calcium sequestration which occurs with compounds structurally-related to glyphosate acid. The NOAEL for glyphosate acid from this study was 10000 ppm (equivalent to 323 mg/kg bw/day for males and 334 mg/kg bw/day for females).

Assessment and conclusion by RMS:
The RMS agrees with the NOAEL of 10000 ppm as proposed by the notifier. This dose level is equivalent to 323 mg/kg bw/day for males and 334 mg/kg bw/day for females. The increased absolute kidney weights at the mid and high-dose in males are not considered adverse as no clear dose-response was shown. No data is provided a relative kidney weights. At the mid and high dose, absolute liver weight was also increased, however, as these changes do not exceed 15% compared with controls this is not considered adverse. At the top dose of 50000 ppm, also an adverse decrease in body weight gain in males and females and a decreased in plasma calcium levels in males was seen.

In the previous assessment in the RAR (2015), the NOAEL proposed by the notifier was agreed and the following was concluded by RMS DE: *"The study is considered acceptable and the NOAEL of 10000 ppm is agreed with. It was noticed that test material of very high purity was used."*

B.6.3.2.18. Oral 13-week toxicity study in dogs – study 4

Data point:	CA 5.3.2/027
Report author	[redacted]
Report year	1996
Report title	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs
Report No	[redacted] 94-0158
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Reticulocytes not counted, clotting not evaluated; blood chloride, sodium and potassium not measured; uterus and thymus not weighed. Deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary
An oral 90–day toxicity study of HR-001 (Glyphosate technical) was conducted in Beagle dogs of both sexes. Groups of 4 males and 4 females were given the test item by incorporating it into a basal diet at a level of 0, 1600, 8000 or 40000 ppm (equivalent to 0, 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females) for a period of 13 weeks. Animals were checked daily for general conditions, mortality and individual food consumption was also measured daily. Body weights were recorded weekly. All animals were subjected to ophthalmology, urinalysis, haematology and blood biochemistry periodically. At termination of treatment, the animals were euthanised and subjected to necropsy and organ weight analysis. Histopathological examination was performed on all animals.

40000 ppm group: Three of the four females showed decrease in urine pH value. However, since the test item is degraded into free acid inducing acidified urine, the decrease in urine pH value without concomitant signs of renal toxicity was not considered of toxicological significance.

8000 and 1600 ppm groups: There were no treatment-related abnormalities in any parameters in either sex.

No significantly adverse effects were observed in Beagle dogs of both sexes following the dietary treatment with HR-001 at a concentration as high as 40000 ppm for 13 weeks

I. MATERIALS AND METHODS

A: Materials

1. **Test material:** Glyphosate technical

Identification: HR-001

Description:	White crystal
Lot/Batch#:	T-950308
Purity:	94.61 %
Stability of test compound:	Not mentioned in the report

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	♂ 5 months; ♀ 6 months
Sex:	Male and female
Weight at dosing:	♂ 27.3 – 32.7 g; ♀ 22.4 – 25.8 g
Acclimation period:	♂ 21 days; ♀50 days
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Filtered and sterilised tap water, *ad libitum*
Housing:	Individually in stainless steel cages 83.5 × 90.0 × 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15/hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-09-20 to 1996-02-08

Animal assignment and treatment:
The test material was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0, 1600, 8000 and 40000 ppm (equivalent to an intake of 0. 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females).

Table 6.3.2-1: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Study design

Test group	Dietary concentration [ppm]	Achieved test concentration [mg/kg bw/day]	Males	Females
Control	0	♂: 0; ♀: 0	4	4
Low	1600	♂: 39.7; ♀: 39.8	4	4
Mid	8000	♂: 198; ♀: 201	4	4
High	40000	♂: 1015; ♀: 1014	4	4

Homogeneity of the test substance in diet was ascertained for all dose levels using the samples taken from the top, middle and bottom portions of the mixer at the first diet preparation (before initiation of the study). The coefficient of variation of the concentrations of technical glyphosate was 2.3 % or less for all test diets and confirmed that the test substance was mixed in the basal diet at good homogeneity.

Concentrations of technical glyphosate in test diets were monitored for all batches of test diets of all dose levels during the study. The overall mean concentrations found in test diets were within a range of 94 –101 % to the nominal levels and confirmed that the test substance was mixed in the test diets at acceptable concentrations.

Mortality

Mortality was expressed weekly as a ratio of the cumulative number of animals found dead or killed in extremis to the effective number of animals per dose group.

Clinical observations
Cage-side observation was performed daily on all animals to detect moribund or dead animals and abnormal clinical signs, and all findings were recorded. In addition, a detailed examination including palpation for masses was performed at least once a week.

Body weight
Body weights of all animals were recorded at initiation of treatment and weekly during the study. In addition, final body weight of each animal was measured before necropsy.

Food consumption and utilisation
Food residues, if any, were collected and weighted every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = \frac{[Feeding\ amount\ (250g\ diet + 250g\ water) - \ food\ residue]}{2}$$

Chemical intake [mg/kg bw/day] was calculated weekly from food consumption and body weight data and the nominal dose level.

Ophthalmoscopic examination
Prior to initiation of treatment and at week 13, all animals were subjected to ophthalmological examinations with a direct ophthalmoscope.

The following parameters were determined: Eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body and fundus.

Haematology and clinical chemistry
Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to haematological examinations. Blood samples were withdrawn with heparinised syringes from the cephalic vein of the animals following overnight starvation. A part of each sample was transferred to a cup treated with EDTA and subjected to the haematological examination.

The following parameters were determined with a fully automated haematology analyser: Haematocrit (Ht), haemoglobin (Hb), erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) and total leukocyte count (WBC).

Prior to initiation of treatment and at weeks 7 and 13, all animals were subjected to biochemical examinations. Plasma from heparinised blood samples from haematological tests was used.

The following parameters were determined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca) and inorganic phosphorus (P).

Urinalysis
Prior to initiation of treatment and at week 13 of treatment, all animals were subjected to urinalysis.
Volume and sediments were determined on urine samples collected for 24 hours using trays. The other parameters were determined on fresh urine samples.

The following parameters were determined: specific gravity, pH, protein, glucose, ketones, occult blood, urobilinogen, bilirubin, appearance urine volume and urinary sediments.

Sacrifice and pathology

All animals were subjected to a complete necropsy and all gross findings were recorded. After 13 weeks of treatment, all animals were anesthetised and euthanised by exsanguinations from the carotid artery before necropsy. At necropsy the organs and tissues except eyes were removed and preserved in neutral-buffered 10% formalin. The eyes were fixed in a phosphate-buffered mixed solution of formalin and glutaraldehyde for about 3 days and transferred to neutral-buffered 10% formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: Brain, heart, adrenals, thyroids with parathyroids, liver, ovaries, kidneys, prostate and spleen.

The following organs and tissues from all animals were histopathologically examined: Brain, spinal cord, peripheral nerve, pituitary, thyroids with parathyroids, thymus, adrenals, tonsil, spleen, bone with marrow, lymph nodes, heart, aorta, tongue, pharynx, buccal mucosa of oral cavity, salivary glands, oesophagus, stomach, liver, gall bladder, pancreas, duodenum, jejunum, ileum, caecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, epididymides, penis, ovaries, oviducts, uterus, vagina, diaphragm, eyes, femoral muscle, skin, mammary gland and all gross lesions.

Statistics
Statistical significance of differences observed in the examination data between the control and treated groups was estimated at 5 and 1% levels of probability. In analyses for body weights, urine specific gravity, urine volume, haematology, blood biochemistry, and organ weights, data were first examined by Bartlett's test for homogeneity of variances among groups . When the variances were homogeneous, the standard one way classification analysis of variance was used to determine whether all the group means were homogeneous or not. When the group means proved to be heterogeneous, Dunnett's (equal number of animals) or Scheff-s (unequal number of animals) multiple comparison test was applied. When it was shown by Bartlett's test that the variances were heterogeneous, the data were evaluated by Kruskal-Wallis 's non-parametric variance test. If significant differences were indicated by this non-parametric procedure, Dunnett's type (equal number of animals) or Scheff-s type mean rank sum test was applied. Urinalysis except data of urine specific gravity and urine volume and food consumption were analysed by MannWhitney's U test. Data on clinical signs, mortality, ophthalmology, necropsy and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any groups during the treatment period.

B. CLINICAL OBSERVATIONS
Statistically significant differences in incidence of clinical signs were not observed between the control and treated groups in either sex. There were sporadic incidences of loose stools in one male in the 8000 ppm group during week 10 and two males in the 40000 ppm group during weeks 5 and 9 for one dog and weeks 5, 6 and 8 for the other dog.

C. BODY WEIGHT
Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no significant changes in food consumption and chemical intake in either sex of the treated groups.

The overall group mean chemical intakes [mg/kg bw/day] over the whole treatment period were calculated from food consumption, body weights and the nominal dose levels. The results are shown in the table below:

Table 6.3.2-2: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Summary of compound intake

Dose level [ppm]	Overall group mean chemical intake [mg/kg bw/day]	
	Males	Females
1600	39.7	39.8
8000	198	201

Table 6.3.2-2: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Summary of compound intake

Dose level [ppm]	Overall group mean chemical intake [mg/kg bw/day]	
	Males	Females
40000	1015	1014

E. OPHTHALMOSCOPIC EXAMINATION

No ocular changes were detected in any dose groups of both sexes.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:

Table 6.3.2-3: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Results of haematological examinations week 13 (mean ± SD)

Parameter	Dose group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
Erythrocyte count (RBC) [10^6/mm^3]	6.73 ± 0.35	↑**7.57* ± 0.44**	↑**7.77** ± 0.23**	↑6.84 ± 0.41	7.20 ± 0.64	↑7.55 ± 0.34	↑7.64 ± 0.46	↑8.02 ± 1.08
Haemoglobin concentration (Hb) [g/dL]	15.2 ± 1.1	↑15.9 ± 0.7	↑15.8 ± 1.0	↓14.8 ± 1.0	15.9 ± 1.0	↑16.8 ± 0.2	↑17.2 ± 0.7	↓14.9 ± 1.3
Haematocrit (Ht) [%]	45.4 ± 3.3	↑47.0 ± 2.1	↑47.6 ± 2.1	↓44.5 ± 3.2	47.3 ± 3.0	↑50.1 ± 0.5	↑51.3 ± 1.8	↓45.8 ± 3.6
Mean corpuscular volume (MCV) [fL]	67.5 ± 1.7	↓62.1 ± 3.0	↓61.3 ± 3.9	↓65.2 ± 4.6	65.9 ± 2.8	↑66.5 ± 2.7	↑67.3 ± 2.8	↓58.1 ± 10.9
Mean corpuscular haemoglobin concentration (MCHC) [g/dL]	33.5 ± 0.2	↑33.8 ± 0.3	↓33.3 ± 0.7	↓33.2 ± 0.9	33.5 ± 0.6	33.5 ± 0.2	33.5 ± 0.3	↓**32.6* ± 0.5**
Lymphocytes (Lym) [10^6/mm^3]	3.6 ± 0.6	↑4.7 ± 1.7	↑3.9 ± 0.5	↓3.5 ± 0.5	3.0 ± 0.9	↑4.1 ± 0.7	↑4.1 ± 1.4	↑3.6 ± 1.0

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose-response relationship was observed. A significant decrease in mean corpuscular haemoglobin concentration (MCHC) observed in females of the 40000 ppm group was considered to be incidental, because the change was also noted for the pre-treatment measurement and was not accompanied with significant abnormalities of erythrocyte count (RBC), haematocrit (Ht) and haemoglobin (Hb).

Blood clinical chemistry

Occasional statistically significant changes in blood biochemistry parameters were observed in the treated groups and are shown in the following table:

Table 6.3.2-4: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Results of clinical chemistry examinations week 13 (mean ± SD)

Parameter	Dose group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
Albumin (Alb) [g/dL]	3.03 ± 0.21	↓2.95 ± 0.24	↑3.07 ± 0.10	↓2.89 ± 0.06	3.02 ± 0.10	↑**3.24* ± 0.06**	↑3.18 ± 0.11	↓2.98 ± 0.10

Table 6.3.2-4: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Results of clinical chemistry examinations week 13 (mean ± SD)

Parameter	Dose group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
Chloride (Cl) [mEq/L]	112.4 ± 1.0	↑**114.1* ± 1.0**	↓112.2 ± 0.9	↑**114.5* ± 0.3**	109.8 ± 0.6	↑109.9 ± 1.0	↓108.8 ± 0.9	↓109.0 ± 1.1
Glucose (Gluc) [mg/dL]	104 ± 4	↓98 ± 7	↓100 ± 2	↓101 ± 3	89 ± 8	↑94 ± 4	↑96 ± 4	89 ± 4

* Statistically significant from controls ($p < 0.05$)

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose response relationship was evident. Although significant increases in chloride (Cl) were observed in males of the 1600 and 40000 ppm groups at week 13, the changes were considered to be incidental because of no dose dependency and their small degrees of alteration.

G. URINALYSIS

In the 40000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis.

There were no significant changes in urinalysis in males and females treated at 8000 ppm or less.

Table 6.3.2-5: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of urinary pH week 13 (number of animals at pH value)

pH	Dose Group [ppm]							
	Males				Females			
	0	1600	8000	40000	0	1600	8000	40000
6.0	-	-	-	-	-	-	-	1
6.5	-	-	-	-	-	-	-	-
7.0	-	-	-	-	-	-	-	1
7.5	-	1	-	1	-	-	-	1
8.0	-	-	-	1	-	-	-	-
8.5	4	3	4	2	4	4	4	1

H. NECROPSY

Organ weights

There were no gross findings with statistically significant differences in incidence and relationship to the treatment in the treated groups of either sex. Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1600 ppm group, the change was considered to be incidental due to the lack of dose-dependency.

Table 6.3.2-6: HR-001: 13-week Subchronic Oral Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of adrenal weight relative to body weight (mean ± SD)

Organ		Dose Group [ppm]							
		Males				Females			
		0	1600	8000	40000	0	1600	8000	40000
Adrenal	Absolute (mg)	906 ± 50	943 ± 121	1061 ± 102	869 ± 136	957 ± 63	1111 ± 104	994 ± 64	996 ± 139
Adrenal	Relative [%]	0.008 ± 0.001	↑0.009 ± 0.001	↑0.010 ± 0.001	0.008 ± 0.002	0.009 ± 0.001	↑**0.011* ± 0.001**	↑0.010 ± 0.001	↑0.010 ± 0.001

* Statistically significant from controls ($p < 0.05$)

Gross pathology

Histopathology
There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.

III. CONCLUSIONS

40000 ppm group: Three of the four females showed decrease in urine pH value. However, since the test item is degraded into free acid inducing a cidified urine, the decrease in urine pH value without concomitant signs of renal toxicity was not considered of toxicological significance.

8000 and 1600 ppm groups: There were no-treatment related abnormalities in any parameters in either sex.

No significantly adverse effects were observed in Beagle dogs of both sexes following the dietary treatment with HR-001 at a concentration as high as 40000 ppm for 13 weeks. It was determined that the no-observable-effect level of HR-001 was 40000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by applicant:
In this study, groups of 4 male and 4 female Beagle dogs were given the test item (glyphosate technical) via the diet at dose levels of 0, 1600, 8000 or 40000 ppm (equivalent to 0, 39.7, 198 or 1015 mg/kg bw/day for males and 0, 39.8, 201 or 1014 mg/kg bw/day for females) for a period of 13 weeks according to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

No toxicologically relevant adverse effects were observed in Beagle dogs of both sexes following the dietary treatment with HR-001 (glyphosate technical) at a concentration as high as 40000 ppm for 13 weeks.

Under the experimental conditions of the study, the NOAEL is considered to be 40000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:
The RMS agrees with the assessment by the notifier and the proposed NOAEL of 40000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively), the highest dose tested as no adverse effects were observed. The tendency towards a lower urinary pH in top dose females was also seen in other studies, however, this is not considered an adverse effect because it is attributed to the acidic properties of the test substance. This conclusion is in line with the previous assessment of this study in the RAR (2015).

In the previous assessment in the RAR (2015), the NOAEL proposed by the notifier was agreed and the following was concluded by RMS DE: "*The study is considered acceptable. The highest dose level of 40 000 ppm is considered the NOAEL because there were no adverse effects of treatment observed. The decrease in urine pH in some high dose females is most likely due to the acidic properties of the test substances and was measured in other toxicological studies before and after, too. Occurring in isolation, without concomitant signs of renal or bladder toxicity, this is not considered an adverse finding.*"

B.6.3.2.19. Oral 13-week toxicity study in dogs – study 5

Data point:	CA 5.3.2/028
Report author	[redacted]
Report year	1985
Report title	Subacute oral toxicity in dogs (for 90 days) of glyphosate (technical) of [redacted]
Report No	Not known
Document No	Not reported
Guidelines followed in study	Not known

GLP	Not known
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Groups of three Mongrel dogs per sex and dose were administered glyphosate (batch and purity not reported); orally via their food at target dose levels of 0 (control group receiving 0.2% agar solution mixed in mutton soup), 100, 250 or 500 mg/kg bw/day for 90 days. In addition, a second group (reversal group) receiving the mid dose of 250 mg/kg bw/day was sacrificed after a 30-day recovery period following treatment. Animals were observed daily for signs of toxicity. Body weight and food consumption were determined regularly. Blood samples for haematological (red and white cell parameters) and clinical chemistry (total serum protein, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, glucose) evaluations were performed at pretest, on study day 46 and on days 91 and 121 just prior to sacrifice. Urinalysis was also performed. All animals were subjected to gross pathological examination and histopathology. Organ weights were determined.
Short description of results:	There were no deaths during the study and no signs of toxicity were observed. Laboratory investigations and pathological examinations did not reveal indications of adverse effects. According to the study authors, alanine aminotransferase was increased in high dose animals, however, the respective mean values were exceptionally high at pretest already. The only findings that could be attributed to treatment were a reduction in body weight gain and, during the second part of the study, a decrease in food consumption. These effects were noted in both sexes but were confined to the highest dose level. In addition, the RMS noted increased absolute and relative liver weights in males at the top dose. Although the liver weight differences compared with controls were not significant, as the changes were >20% these are considered treatment-related and adverse. Thus, the mid dose of 250 mg/kg bw/day was considered the NOAEL in this study.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supportive only due to reporting deficiencies. For examples, the year when the study was performed was not indicated in the original report, test substance purity not reported, statistical analysis of the results was not reported. RAR (2015): The study was considered invalid due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Therefore and since the study report is not available to GRG, this study is not considered to be reliable by GRG. **Conclusion GRG:** The study is considered unacceptable, category 4b. **Conclusion AGG**: The study report has been made available to AGG by BVL. The RMS has evaluated the study and agrees with the previous conclusion that the study is not considered acceptable due to missing information on the batch and purity of the test substance and verification of the amount of test substance in the test diet (stability, homogeneity, actual concentration). The RMS agrees with the results reported above and with the conclusion that the treatment-related effects were confined to a decreased body weight and a decreased food consumption in both sexes and an increased absolute and relative liver weight in males at the top dose of 500 mg/kg bw/day. However, no NOAEL is proposed as the study is not considered acceptable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. The former RMS (BVL) has made the study report available to the current RMS.

B.6.3.2.20. Oral 6 month toxicity study in dogs – study 6

Data point	CA 5.3.2/029
Report author	[redacted]
Report year	1983
Report title	Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs
Report No	810166
Document No	Not reported
Guidelines followed in study	No guideline statement, but in general accordance with OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998; OECD 452, 2018)	Blood chloride and urine volume were not measured, unclear if a middle section of the spinal cord was observed microscopically. Deviations from the current version of OECD 409 (1998) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 409.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary

MON-0139 (aqueous solution of the isopropylamine salt of glyphosate) was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 10, 60 or 300 mg/kg bw/day for approximately six months.

Clinical observations were done twice daily. Weekly determinations of individual body weight and daily food consumption were performed. Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals. Haematological and blood biochemistry parameters were evaluated prior to start of treatment and monthly during treatment. Urinalysis was performed pre-test and during months 2, 4 and 6 of treatment. At the end of the scheduled dosing period, the animals were sacrificed and subjected to a full examination post mortem. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. No unusual changes occurred in food consumption or clinical signs as a result of MON-0139 exposure. However, mean body weights were decreased by >10% compared with controls in males at the end of the study (day 197), which is considered treatment-related and adverse by the RMS. The only change which potentially related to MON-0139 administration was an elevation of serum alkaline phosphatase levels in males and females at all sampling intervals. There was no indication of the source of the increased levels of this enzyme as no microscopic evidence of lesions in the organs usually responsible for elevations in serum alkaline phosphatase levels were found. This, together with the generally small magnitude of these elevations and the lack of dose response in females makes the interpretation of this change equivocal as regards biological significance and correlation with MON-0139 administration. Changes in total LDH and LDH isoenzymes were identified but the occurrences were erratic. Total LDH levels were depressed in males especially from higher dosage levels at 4, 5 and 6 months of treatment primarily from decreases in LDH 5. Other changes in LDH isoenzymes included mild elevations of LDH 2 and LDH 3 in males and decreases of these isoenzymes in females. This lack of consistency in the direction of change between males and females and a lack of consistent response from one sampling period to another, along with the lack of correlation of these changes with microscopic lesions or other serum chemistry changes, suggested an unlikely relationship to MON-0139 exposure. Other changes in serum chemical and changes in haematological and urinalysis parameters were isolated events and/or were within the range of normal values and were not considered related to administration of the test substance. *Post mortem*, there were no gross lesions, organ weight changes or microscopic findings that were considered associated with administration of MON-0139.

The RMS considers the decreased body weight at the end of the study at the top dose (300 mg/kg bw/day) in males as treatment-related and adverse. Based on this observation, the proposed NOAEL is 60 mg/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON-0139 (aqueous solution of the isopropylamine salt of glyphosate)
Description:	Amber liquid
Lot/Batch#:	LURT-12011
Purity:	62.49% for isopropylamine salt of glyphosate
Stability of test compound:	Shown stable during the dosing period

2. Vehicle and/or positive control: Empty gelatin capsule

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.3 – 10.6 kg; ♀ 5.4 – 8.1 kg
Acclimation period:	Approx. 6 weeks
Diet/Food:	Purina Certified Dog Chow® 5007 limited to a 2 – 3 hour period
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel dog cages
Environmental conditions:	Temperature: 20 – 22 °C (68 – 72 °F) Humidity: Not reported Air changes: Not reported, assume standard 12 hours light/dark cycle

B: Study design and methods

In life dates: 1982-01-26 to 1982-08-13

Animal assignment and treatment:
Six male and six female dogs per dose level received MON-0139 orally, by gelatin capsule administration, once daily for approximately 6 consecutive months. The liquid MON-0139 was placed into empty gelatin capsules (size 1/8 oz.). Doses were adjusted weekly to correspond with each animal's body weight. Capsules were prepared daily. Each dog received one capsule approximately one to four hours after removing its food each day. Control animals were each administered one empty gelatin capsule daily. Animals were acclimated to the laboratory for approximately 6 weeks and passed a veterinary health check prior to assignment to the study. Animals were individually housed in stainless steel dog cages.
Analyses after the completion of the study indicated 63.2% MON-0139 compared to an assay (conducted prior to the study) provided by the sponsor of 62.49% MON-0139. This +0.7 % variation of analyses was within normal limits and no decomposition of MON-0139 were demonstrated.

Table 6.3.2-1: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs [redacted] 1983): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	6	6
Low	10	6	6
Mid	60	6	6
High	300	6	6

Mortality
Each animal was checked for mortality or signs of morbidity at least twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made at least twice daily (morning and afternoon) on all animals.

Body weight
Weekly determinations of individual body weight were performed.

Food consumption and utilisation
Daily determinations of food consumption were performed.

Ophthalmoscopic examination
Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals.

Haematology and clinical chemistry
Laboratory investigations of haematology and clinical chemistry were performed after overnight fasting on all the dogs before dosing started and approximately monthly during the treatment period. The blood samples were taken from the jugular vein after an overnight fast.

EDTA was used as an anti-coagulant for evaluation of all haematology parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time.

For clinical chemistry evaluations, serum was harvested from whole blood and analysed for the following parameters: Blood urea nitrogen, glucose, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactic dehydrogenase (total and isozyme determination), sodium, potassium, calcium, creatinine, total protein, albumin, globulin (calculated), cholesterol, phosphorus, direct and total bilirubin.

Urinalysis
Urinalysis was performed on all the dogs before dosing started and again during months 2, 4, and 6 of treatment. Urine was collected using metabolism cages. The following parameters were measured: Appearance, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed.

Sacrifice and pathology
After 6 months of consecutive treatment, all surviving animals were fasted overnight, sacrificed by intravenous sodium pentobarbital followed by exsanguination and subjected to a gross pathological examination. Terminal body weights were recorded immediately prior to sacrifice.

The following organ weights were determined: Adrenals, thyroids with parathyroids, pituitary, brain, heart, liver, kidneys, testes with epididymides and ovaries.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aorta, brain (cerebrum, cerebellum, medulla), colon, duodenum, epididymides, eyes with optic nerve,

heart, ileum, jejunum, kidneys, liver, gallbladder, lungs, lymph nodes (mesenteric), mammary gland, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rib with marrow, salivary gland (mandibular), spinal cord (cervical, lumbar), sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroids, trachea, urinary bladder, and uterus. All tissues above from the control and high dose groups were examined histopathologically. Only tissues with gross lesions were microscopically observed from the mid and low dose groups as no target organs were identified in the high dose animals.

Statistics
Non-categorical data from haematological, serum chemical and urinalysis were statistically examined by Dunnett's test for the comparison of multiple treatments with a control, and/or by inspection. Categorical data were examined to determine any remarkable group differences. Statistical evaluation of differences in body weights, food consumptions, terminal body weights and absolute organ weights between treated and control groups was accomplished by the use of Dunnett's test. The Mann-Whitney test with Bonferroni's Inequality was used to assess the organ/body weight ratios. A comparison of the frequency of microscopic lesions between treated groups and controls was evaluated by the use of Fisher's Exact Test with Bonferroni's Inequality.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No unusual clinical signs were noted that could be attributed to MON-0139 exposure.

C. BODY WEIGHT
No significant body weight changes occurred between any of the dose groups. However, mean body weights were decreased by >10% compared with controls in males at the end of the study (day 197), although not significant, this is considered treatment-related and adverse by the RMS.

Table 6.3.2-2: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs [redacted] 1983): Intergroup comparison of body weights at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	10	60	300	0	10	60	300
Body weight								
Pre-test[a]	8.5	8.6	8.6	8.7	6.5	6.6	6.6	6.5
1 month (day 29)	9.3	9.3	9.3	9.4	6.9	7.1	7.2	7.1
3 months (day 92)	10.8	10.6	10.7	10.4	7.9	7.8	7.9	7.7
6 months (day 197)	12.0	11.4	11.8	10.4 (-13%)	9.2	8.2	8.6	9.0

[a] Mean for all animals on study by sex; * Statistically significant from controls ($p < 0.05$);

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no treatment-related effects. Food consumption in treatment groups was comparable to controls. Test compound was administered by gelatine capsule with dosages adjusted according to individual animal weight. Determination of the degree of absorption of the test item following dosing was not performed.

E. OPHTHALMOSCOPIC EXAMINATION
There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
Statistically significant changes were observed in several haematological parameters. However, the only parameter in which changes occurred in the same sex at several different sampling periods was mean cell haemoglobin concentration (MCHC). As changes were very slight and no dose-response is being observed, these changes are not considered relevant.

Table 6.3.2-3: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs ([redacted] 1983): Intergroup comparison of MCHC at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	10	60	300	0	10	60	300
MCHC [g/dL]								
Pre-test[a]	34.3	-	-	-	34.3	-	-	-
1 month	33.6	↑**34.2***	↑**34.2***	↑**34.2***	33.8	↑**34.2***	↑**34.3****	33.8
3 months	33.3	↑33.8	↓33.2	↑33.6	33.4	↑33.7	↓33.1	33.4
6 months	31.9	↑**32.5****	↑**32.6****	↑**32.8****	32.1	↑32.4	↑**32.6***	↑**32.7****

[a] Mean for all animals on study by sex;
* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Blood clinical chemistry

Statistically significant changes were observed in several serum chemistry parameters. The only parameters in which changes occurred in the same sex at several different sampling periods were total LDH and LDH isoenzymes. As no clear pattern is seen over time for each sub-enzyme and between sexes, these changes are not considered relevant. Further, these changes were not accompanied by gross or microscopic liver lesions.

Alkaline phosphatase (ALP) levels were generally slightly elevated in both males and females at all sampling periods, although it was statistically significant only in males from Group 3 at 5 months of treatment. This change in males was most evident at the highest dosage level while in females it was usually present among all treated groups often with no dose response. This effect was considered as possibly related to administration of the test substance. Changes in other clinical chemistry parameters had no biological significance and were apparently unrelated to compound administration. As changes are less than 50% compared with controls, there changes are not considered relevant by the RMS.

Table 6.3.2-4: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs [redacted] 1983): Intergroup comparison of specific clinical chemistry parameters at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	10	60	300	0	10	60	300
LDH (total) [IU/L]								
Pre-test[a]	107	-	-	-	147	-	-	-
1 month	168	↑183	↓134	↓125	103	↑**183****	↑121	↓80.8
3 months	70.3	↓**42.8****	↓61.3	↓62.5	83.6	↓80.1	↑89.5	↓53.7
6 months	76.8	↓57.6	↓**45.0****	↓**44.5****	32.9	↑40.4	↑43.3	↑40.3
LDH 1 [%]								
Pre-test[a]	24	-	-	-	17	-	-	-
1 month	15	↓**7****	15	15	22	↓17	22	↑23
3 months	29.3	↓26.3	↓20.3	↑31.0	24.8	↑30.7	↑26.7	↑31.5
6 months	42.3	↓38.2	↓33.5	↓41.2	36.0	↑41.0	↑39.5	↓32.8
LDH 2 [%]								
Pre-test[a]	24	-	-	-	23	-	-	-
1 month	18	↓15	18	↓17	22	↓**15****	↓18	↑23
3 months	18.0	↑**22.3***	↑**22.7***	↓15.8	24.0	↓**17.2****	↓**17.2****	↑24.2
6 months	16.0	↑19.8	↑**22.3***	↑**21.7***	23.0	↓20.5	↑23.8	↓23.3
LDH 3 [%]								
Pre-test[a]	26	-	-	-	30	-	-	-
1 month	23	↓20	↑**30***	↑26	27	↓**23***	↓**23***	↓26
3 months	25.2	↑**30.7***	↑27.7	↓20.5	30.2	↓**21.3****	↓**23.0***	↓**21.3****
6 months	22.7	↑22.8	↑27.8	↑24.0	30.0	↓27.0	↓26.8	↑31.7
LDH 4 [%]								

Table 6.3.2-4: Six Month Study of Mon 0139 by Gelatin Capsule to Beagle Dogs [redacted] 1983): Intergroup comparison of specific clinical chemistry parameters at selected time periods (group means)

Parameter	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	**10**	**60**	**300**	**0**	**10**	**60**	**300**
Pre-test[a]	15	-	-	-	17	-	-	-
1 month	21	21	↓20	↑22	17	↑20	↑19	↓**14***
3 months	13.8	↓12.8	↓13.2	↓13.7	13.7	↓12.8	↑15.2	↓11.2
6 months	12.2	↑13.0	↑12.7	↓9.3	9.7	↓7.5	↓8.7	↑10.1
LDH 5 [%]								
Pre-test[a]	11	-	-	-	13	-	-	-
1 month	23	↑**38****	↓17	↓20	12	↑**26****	↑19	↑**14***
3 months	13.7	↓9.2	↑16.2	↑19.0	7.3	↑**18.0****	↑**18.0****	↑11.8
6 months	7.5	↓6.2	↓3.7	↓3.8	1.7	↑4.0	↓1.3	↓1.0
ALP [IU/L]								
Pre-test[a]	383	-	-	-	351	-	-	-
1 month	345	↓343	↑347	↑382	273	↑295	↑330	↑302
3 months	238	↑245	↑251	↑285	246	↑289	↑300	↑263
5 months	146	↑151	↑156	↑**205****	152	↑235	↑233	↑207
6 months	137	↑169	↑157	↑177	152	↑205	↑191	↑210

[a] Mean for all animals on study by sex;
* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

Statistically significant changes were observed in several urinalysis parameters. However, there were no parameters in which changes occurred in the same sex at several different sampling periods.

H. NECROPSY

Organ weights

The only mean absolute organ weight value that was statistically different from the control value was a decreased mean thyroid gland weight in males from the intermediate dosage group. This change was not present in the highest dosage group, therefore, it was not considered to have resulted from MON-0139 administration. The mean right testicular weight of the highest dosage group was increased (not statistically significant) due to an increase in the recorded testicular weight of one animal. There were, however, no gross or microscopic lesions recorded for the right testis of that animal. The only changes in organ to body weight ratios were increases in heart and thyroid weights relative to terminal body weights. The increased relative heart weights occurred in females from the intermediate dosage level and the increased relative thyroid weights occurred in females from the lowest dosage level, both of which resulted from non-significant increases in the absolute organ weights that were apparently unrelated to chemical administration.

Gross pathology

There were no gross lesions that were associated with administration of MON-0139.

Histopathology

There were no microscopic lesions that were associated with administration of MON-0139.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. No unusual changes occurred in body weight, food consumption or clinical signs as a result of MON-0139 exposure. The only change which potentially related to MON-0139 administration was an elevation of serum alkaline phosphatase levels in males and females at all sampling intervals. There was no indication of the source of the increased levels of this enzyme as no microscopic evidence of lesions in the organs usually responsible for elevations in serum alkaline phosphatase levels were found. This, together with the generally small magnitude of these elevations and the lack of dose response in females makes the interpretation of this change equivocal as regards biological significance and correlation with

MON-0139 administration. Other changes in serum chemical and changes in haematological and urinalysis parameters were isolated events and/or were within the range of normal values and were not considered related to administration of the test substance. *Post mortem*, there were no gross lesions, organ weight changes or microscopic findings that were considered associated with administration of MON-0139.

Assessment and conclusion by applicant:
In this study, the test item MON-0139 (aqueous solution of the isopropylamine salt of glyphosate) was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 10, 60 or 300 mg/kg bw/day for approximately six months according to a testing regime similar to OECD 409 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

All animals survived until termination of the study. There were no clinical signs of toxicity. There were no effects on body weight, food consumption, ophthalmoscopy, haematology, urinalysis, gross postmortem findings, organ weights or microscopic pathology considered biologically adverse or related to administration of MON-0139. An apparent increase in alkaline phosphatase activity potentially associated with treatment was observed in top dose males from the second measurement onwards reaching statistical significance in month five only. Changes in total LDH and LDH isoenzymes were identified but the occurrences were erratic. No other clinical chemistry parameters were considered adversely affected. As the increase in alkaline phosphatase activity was not accompanied by any other indication of organ or tissue damage, the highest dose of 300 mg/kg bw/day can be considered the NOAEL.

Assessment and conclusion by RMS:

The RMS agrees with the assessment by the applicant. However, the RMS considers the decreased body weight at the end of the study at the top dose (300 mg/kg bw/day) in males as treatment-related and adverse. Based on this observation, the proposed NOAEL for MON0139 is 60 mg/kg bw/day.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"*There is an acceptable study in which the formulation MON0139 (62.49 % IPA salt of glyphosate) had been administered for six months in gelatine capsules to Beagle dogs* [redacted] *1983, TOX9552361). This study is still included since it was found acceptable upon re-evaluation by RMS. At least, it is suitable to show that this salt proved to be of no higher toxicity in dogs than the acid*."

B.6.3.2.21. Oral 13-week toxicity study in dogs – study 7

Data point	CA 5.3.2/030
Report author	[redacted]
Report year	1981 (Hungarian report, Revised and English version 1991)
Report title	3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs
Report No	8011
Document No	Not reported
Guidelines followed in study	None indicated, study preceded OECD 409 guideline, but the study design was similar to OECD 409 (1981)
Deviations from current test guideline (OECD 409, 1998)	Not all required haematological, clinical chemistry, urinalysis parameters were evaluated; some organs were not weighed or microscopically examined. Formulated diets were not analysed for concentration, homogeneity or stability. Purity of the test substance not stated in the revised report since the respective supplement to the original report was missing to the author of the revised report. Individual and group data not reported for body weight, food consumption, haematology, clinical chemistry and organ weights.
Previous evaluation	Not accepted in RAR (2015)

GLP/Officially recognised testing facilities	No, GLP was not compulsory when study was performed
Acceptability/Reliability	**Conclusion GRG**: Supportive, category 3a **Conclusion AGG**: The study is considered unacceptable due to serious reporting deficiencies, e.g. the purity and manufacturer of the test substance is not reported and concentration, homogeneity and stability of the test substance was not verified in the test diet and reporting tables of body weight, food consumption, haematology, clinical chemistry and organ weights are missing. Further, several haematological, clinical chemistry and urinary parameters were not evaluated (refer to mentioned deviations above).

Executive Summary
This is a revised and English report version of the 1981 Hungarian report. The revision was regarded as necessary by the study sponsor in order to eliminate slight deficiencies and to analyse more deeply the obtained experimental data. For this revision, none of the original data of the study were modified or omitted according to the best of the revision author's knowledge.

Glyphosate (purity and manufacturer not stated since the respective supplement as mentioned in the original study report was not included in the revised report) was administered to groups of four male and four female Beagle dogs for three months via food at target dietary concentrations of 0, 200, 600 or 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day).

All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were overall not affected (values not reported in the revised report). Haematology, clinical chemistry, urinalysis and gross pathological examination did not reveal adverse effects (values not reported in the revised report). Liver weight (values not reported in the revised report) was marginally lower at the highest dose level (2000 ppm). In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group.

As the study is not considered acceptable no NOAEL is being proposed.

II. MATERIALS AND METHODS

A: Materials

1. **Test material:** Glyphosate
   - Identification: 14/980
   - Description: Not reported
   - Lot/Batch#: 03090380
   - Purity: Purity not stated since the respective supplement mentioned in the original study report was not provided in the revised report
   - Stability of test compound: Not reported
2. **Vehicle and/or positive control:** Plain diet / none
3. **Test animals:**
   - Species: Dog

Strain:	Beagle
Source:	[redacted]
Age:	9 – 11 months
Sex:	Male and female
Weight at dosing:	Body weight data at test start not included in revised report
Acclimation period:	3 weeks
Diet/Food:	Composition not provided in the revised report.
Water:	Tap water, *ad libitum*
Housing:	Individually in kennels (1 square meter)
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: 50 ± 5 % Air changes: 5-7 /hour Light/dark cycle: Not reported

B: Study design and methods

In life dates: 1980-11-24 to 1981-02-26

Animal assignment and treatment:
The test material was offered daily in diet to groups of 4 males and 4 females Beagle dogs for 13 weeks. Target dietary concentrations were 0, 200, 600 or 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day). Food was mixed with appropriate amounts of test substance one week before the start of the study and monthly thereafter. Food was provided to the animals for 3 hours each day between 0900 and 1200 hours and unconsumed food was weighed and food consumption calculated weekly. The report does not indicate that formulated diet was analysed for concentration, homogeneity or stability. The study design is summarised in the table below:

Table 6.3.2-1: 3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs [redacted] 1981): Study design

Test group	Dietary concentration [ppm]	Males	Females
Control	0	4	4
Low	200	4	4
Mid	600	4	4
High	2000	4	4

Mortality
Animals were observed daily.

Clinical observations
Animals were observed daily.

Body weight
Body weight measurement was done weekly.

Food consumption and utilisation
Each day, unconsumed food was weighed and food consumption calculated weekly. Chemical intake [mg/kg bw/day] was calculated weekly from food consumption and body weight data and the nominal dose level.

Ophthalmoscopic examination
Not performed.

Haematology and clinical chemistry

Blood samples were collected from the antebrachial vein prior to initiation of treatment and on study days 42 and 85±2 days. The following haematology parameters were measured: haematocrit, haemoglobin, erythrocyte count, and total and differential leukocyte counts.

The following clinical chemistry parameters were measured: Alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, creatinine, blood urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, glucose, bilirubin, potassium and sodium.

Urinalysis
Urine was collected using metabolic cages at termination of the dosing period. The following urine parameters were evaluated: specific gravity, pH, protein, glucose and microscopic examination of sediment was performed.

Sacrifice and pathology
After 13 weeks of treatment, all animals were subjected to a complete necropsy and all gross findings were recorded. Animals were anaesthetised with phenobarbital and euthanised by exsanguinations from the carotid artery before necropsy. At necropsy, the organs and tissues were removed and preserved in 8 % formalin.

Weights of the following organs were recorded for all animals and the ratios to the final body weight were calculated: Brain, hypophysis, pituitary, heart, adrenals, thyroids, liver, gonads, and kidneys.

The following organs and tissues from all animals were examined microscopically: Brain (cerebrum, cerebellum pons), hypophysis, spinal cord, peripheral nerve, pituitary, thyroids, adrenals, spleen, lymph nodes (cervical, mesenteric), heart, aorta (thoracic, abdominal), salivary gland (submandibular), oesophagus, stomach (cardiac, fundus, pylorus), liver, gall bladder, pancreas, duodenum, jejunum, ileum, large intestine, trachea, lungs, kidneys, urinary bladder, testes, prostate, ovaries, uterus, eyes, femoral muscle, and all gross lesions.

Statistics
Differences between the control and treated groups were analysed using Student's two samples t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
All animals survived the study.

B. CLINICAL OBSERVATIONS
No signs of toxicity were observed during the study.

C. BODY WEIGHT
There were no statistically or biologically significant differences in weight variations occurring during the study between control and glyphosate treated animals. The supplement containing the data was not included in the revised study report.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There was an isolated reduction in food consumption in some of the high dose (2000 ppm) group animals at or around study week 9 primarily associated with 3 dogs. Reduced food intake occurred at other times during the study similarly in controls and treated dogs but these reductions were no longer observed by the end of the study period. However, food intake was reduced in all study groups at the end of the dosing period causing a moderately lower substance intake. The supplement containing the food consumption data was not included in the revised study report.

The overall group mean chemical intakes [mg/kg bw/day] over the whole treatment period were calculated from food consumption, body weights and the nominal dose levels. The results are shown in the table below:

Table 6.3.2-2: 3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs [redacted] 1981): Summary of mean group compound uptake, selected weeks [mg/kg bw/day]

Study Week	Dose levels for both sexes combined [mg/kg bw day]			
	Dose group [ppm]			
	0	200	600	2000
1	-	9.07	25.9	83.85
3	-	10.54	25.86	91.88
6	-	10.95	24.73	88.90
9	-	9.86	28.31	68.41
10	-	8.14	25.78	64.54
11	-	6.26	21.98	71.49
12	-	7.28	19.66	50.93
13	-	4.58	15.18	44.73
Weeks 1 – 13	-	9.08	24.92	77.43

E. OPHTHALMOSCOPIC EXAMINATION

Not performed.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no significant differences in haematology values in measured parameters between control and treated dogs during the study. The supplement containing the data was not included in the revised study report.

Blood clinical chemistry

There were no significant differences in clinical chemistry values in measured parameters between control and treated dogs during the study. The supplement containing the data was not included in the revised study report.

G. URINALYSIS

There were no significant differences in clinical chemistry values in measured parameters between control and treated dogs during the study. The supplement containing the data was not included in the revised study report.

H. NECROPSY

Organ weights

Marginally lower absolute liver weights resulted in lower relative liver weights for high dose animals. There were no differences in weights of the other organs between controls and treated animals. The supplement containing the data was not included in the revised study report.

Gross pathology

The revised report indicates that the findings at necropsy were rare, slight and nonspecific to treatment with glyphosate with no differences observed between control and treated groups. The report indicates a "weaker general condition of the animals at necropsy" that did not correlate with the clinical laboratory and pathology findings. One 600 ppm group female and one 2000 ppm group female were graded as in poor general condition at necropsy.

Histopathology

No findings considered indicative of severe toxicity were observed in any of the organs examined. Alterations considered mild were found only in the livers of some higher dose group dogs that were described as "indistinct structure" that did "not transfer to degeneration and was regarded as fully reversible". The revised report presents a histopathology report indicated to be prepared from the primary histopathology findings from 1981. The changes observed in the livers of the high dose group dogs (2000 ppm) were described in the revised report as round shaped, enlarged hepatocytes and occasionally narrowing of some of the hepatocytic trabeculae and slight dissociation of the structure. Non-pathologic congestions seen the liver of one lower dose animal was more pronounced in the animals with "indistinct liver structure". The abnormal presence or localisation of connective tissue or fibrosis were not observed. No other changes observed were considered attributable to treatment with glyphosate. It was concluded that treatment-related findings occurred only in the liver of the high dose group animals in the form of increased congestion and "indistinct structure". These liver lesions were considered functional and reversible. No other findings were considered of toxicological or pathological significance.

Table 6.3.2-3: 3 Month Oral Dietary Toxicity Study with Glyphosate in Dogs [redacted] 1981): Summary incidence of liver histopathological findings

Finding	Dietary Concentration [ppm]							
	Males				Females			
	0	200	600	2000	0	200	600	2000
Focal subacute inflammation	4/4	1/4	2/4	3/4	4/4	2/4	1/4	3/4
Congestion	0/4	1/4	0/4	3/4	0/4	0/4	0/4	4/4
Indistinct structure	0/4	0/4	2/4	2/4	0/4	0/4	1/4	4/4

III. CONCLUSIONS

All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were overall not affected. Haematology, clinical chemistry, urinalysis and gross pathological examination did not reveal adverse effects. Liver weight (values not reported in the revised report) was marginally lower at the highest dose level (2000 ppm). In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group.
None of the parameters recorded or measured revealed treatment related changes, except histopathology, where more or less functional type morphological changes were described in the livers of some dogs of the highest dose group.

Assessment and conclusion by applicant:
Groups of four male and four female Beagle dogs were treated for three months with glyphosate via their food at target dietary concentrations of 0, 200, 600 or 2000 ppm (equivalent to 0, 9.08, 24.92 or 77.43 mg/kg bw/day). All animals survived the dosing period and there were no clinical signs of toxicity. Body weight and food consumption were not affected. Haematology, clinical chemistry, urinalysis and gross pathological examination did not reveal adverse effects. Liver weight (value not reported in the revised report) was marginally lower at the highest dose level (2000 ppm). In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three male and all female dogs in the highest dose group. The toxicological significance of these findings is equivocal. Based on these results, an average daily glyphosate intake of approximately 25 mg/kg bw/day calculated from the

600 ppm mid-dose target concentration can be considered a NOAEL for dogs under the conditions of this study.

Assessment and conclusion by RMS:

The study is considered unacceptable due to serious reporting deficiencies (refer to acceptability/reliability comments by RMS above). This is in line with the previous assessments in the DAR and RAR (2015).

In the study report, only the histopathology results were adequately reported. In two high dose males and in all high dose females, a histopathological feature called "indistinct structure" in the liver was described. This was also seen at the mid dose (600 ppm) level in a smaller number of dogs (2 males and one female). This change was characterised by round shaped and enlarged hepatocytes and occasionally also by the narrowing of some of the hepatocytic trabeculae and slight dissociation of the liver structure. In addition, congestion of the liver was noted in three males and all female dogs in the highest dose group. As already indicated in the DAR, it should be taken into account that similar liver effects were also seen in the 12-month dog study from the same laboratory (refer to B.6.3.2.26) but were not seen in any other dog study with glyphosate obtained from other manufacturers.
As the study is not considered acceptable no NOAEL is being proposed.

B.6.3.2.22. Short term dog - one-year oral – study 1

Data point	CA 5.3.2/031
Report author	[redacted]
Report year	2007
Report title	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs
Report No	29647[redacted]
Document No	Not reported
Guidelines followed in study	OECD 452 (1981); JMAFF 2-1-14 (2001)
Deviations from current test guideline (OECD 452, 2018)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, category 2a **Conclusion AGG**: The study is considered acceptable.

Executive Summary

The toxicity potential of glyphosate technical was assessed in a 1-year oral toxicity study in male and female Beagle dogs. Groups of four dogs per sex received daily doses (capsules) of 0, 30, 125, or 500 mg/kg bw/day for 52 consecutive weeks (dose level selection was based on the results of a 13-week study run in the same laboratory). Observations covered mortality, clinical signs, body weight, food consumption, ophthalmological examinations, haematology, clinical chemistry, urine analysis, organ weights, necropsy and histopathological examination.

No unscheduled deaths or premature sacrifices occurred during the study. There were no treatment-related effects on clinical signs, eyes, body weight, food consumption, haematology, clinical chemistry or urine analysis parameters in both sexes. However, a reduced body weight gain was noted in males at 500 mg/kg bw/day, which was considered treatment-related and adverse by the RMS. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects.

The RMS proposes a NOAEL of 125 mg/kg bw/day based on these effects on body weight gain in males dosed at 500 mg/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

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

2. Vehicle and/or positive control: Gelatine capsules size 12 (Torpac, New York, USA) / none

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Males and females
Weight at dosing:	7.8 – 8.9 kg (males); 7.2 – 7.9 kg (females)
Acclimation period:	13 days + 20 days pre-treatment period
Diet/Food:	125 C3 pelleted diet (SAFE, Villemoisson, Epinay-sur-Orge, France), approx. 300 g per day. Due to weight loss in three animals the amount for these dogs was increased to 350 g/day from day 149, 180, and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards
Water:	Tap water, *ad libitum*
Housing:	Individually in pens containing wood shavings for bedding, except when a urine sample was required. The dogs were group-housed once a week, by sex and dose group, after the last recording of clinical signs in the afternoon, until the next morning
Environmental conditions:	Temperature: 20 ± 5 °C Humidity: 50 ± 20 % Air changes: approx. 12 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 2005-09-27 to 2006-10-17

Animal assignment and treatment:
In a chronic oral toxicity study groups of four Beagle dogs per sex received daily doses of 0, 30, 125 or 500 mg/kg bw/day glyphosate technical in gelatine capsules for 52 consecutive weeks. The dose levels were selected based on results of a 13-week oral (capsule) toxicity study in dogs. Dose formulations were prepared weekly by adding the required amount to the capsules. The dosages were calculated based on minimum nominal active substance content of 950 g/kg glyphosate in the test item. Analyses of the test item showed a glyphosate content consistently above 95 %. Thus, no adjustment was considered necessary. Since the test item was added under GLP conditions, no additional analyses of dose formulations were deemed necessary.

Administrations of dose capsules were done approximately the same daily time each day. The low and mid-dose animals received one capsule per day, the high-dose and control dogs received three capsules per day. The quantity of dosage form applied to each animal was adjusted weekly based on the most recently recorded body weight.

Clinical observations
Observations for morbidity, and mortality were made twice daily. A check for clinical signs of toxicity was made at least once daily on all animals. In addition, a detailed clinical examination was performed once before start of treatment and weekly thereafter until termination.

Body weight
Individual body weights were recorded three times before group allocation, on day 1 (prior to treatment) and weekly thereafter during the conduct of study and at termination.

Food consumption and compound intake
Food consumption of each animal was estimated daily by noting the difference between the amount provided and the remaining amount on the next morning. Food consumption was expressed as percentage of quantity provided. Whenever fasting was required, food was removed at the end of the day and estimation of food consumption was made at that time.

Ophthalmological examination
Ophthalmological examinations were performed on all dogs prior to start and at the end of the treatment period. Pupillary light and blink reflexes were evaluated first. Mydriasis was then induced by adding Tropicamide solution into the eyes and the appendages, optic media and fundus were examined by indirect ophthalmoscopy.

Haematology and clinical chemistry
Blood samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. The following haematological parameters were examined: Haemoglobin concentration (HB), erythrocyte count (RBC), mean cell volume (MCV), packed cell volume (PCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), thrombocytes (PLAT), leukocytes (WBC), differential white cell count with cell morphology, neutrophils (N), eosinophils (E), basophils (B), lymphocytes (L), monocytes (M), reticulocytes (RETIC), prothrombin time (PT) and activated partial thromboplastin time (APTT). The following clinical chemistry parameters were examined: Alkaline phosphatase (ALP), alanine aminotransferase activity (ALAT), aspartate amino transferase (ASAT), albumin, albumin/globulin ratio, total bilirubin, glucose, urea, calcium, chloride, total cholesterol, creatinine, γ-glutamyl-transferase (GGT), inorganic phosphorus, total protein, sodium, potassium and triglycerides.

Urinalysis
Individual urine samples were collected from all dogs prior to treatment, in week 25 and at the end of the treatment period in week 51. For sampling dogs were fasted overnight for at least 14 hours. Urine was collected in the presence of thymol crystals. The following examinations were made: Appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood and urobilinogen. The sediment was examined microscopically for leukocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and cells.

Sacrifice and pathology
All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weighed: Adrenals, brain (including medulla/pons, cerebellar and cerebral cortex), epididymides, heart, kidneys, liver, spleen, thymus, uterus (horns and cervix), pituitary, prostate, ovaries, testes and thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): Adrenals, aorta, brain (including medulla/pons, cerebellar and cerebral cortex), caecum, colon, duodenum, oesophagus, eyes and optic nerve, epididymides, femur with articulation, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs with bronchi, mammary gland, mandibular lymph node, mesenteric lymph node, skeletal muscle, optic nerve, ovaries, oviducts, parathyroid, pancreas, pituitary, prostate, rectum, salivary glands (parotid and submandibular), skin, spinal cord (cervical, thoracic and lumbar), spleen,

sternum with bone marrow, stomach, sciatic nerve, testes, thymus, thyroids with parathyroid, tongue, trachea, urinary bladder, ureters, uterus (horns and cervix) and vagina.

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics
Statistical analysis of body weight, haematology, blood biochemistry, urinalysis and organ weight data was done according to the statistical decision tree shown in "*Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*" (OECD, 2002), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortalities or premature sacrifices occurred during the treatment period.

B. CLINICAL OBSERVATIONS
There were no treatment-related clinical signs observed during the study period.

Observed clinical signs consisted of vomiting or soft faeces, thin appearance, hyperactivity, ptyalism, skin findings (scabs and erythema, generally localized on the ear(s)) and nodules on the ears. These clinical observations were seen transiently, and were encountered with a similar incidence in both control and treated animals and/or were independent to the administered dose-level and/or were already present before the beginning of the treatment period.

C. BODY WEIGHT
There was no treatment-related effect on body weight development. The lower mean body weight recorded in high dose males at the end of the treatment period was due to the lower mean body weight gain during the first month of the study (see Table below). Individual body weight changes were within the range of physiological variations. In addition, such body weight changes were observed in both control and treated dogs. The RMS considers the reduced body weight gain in males at 500 mg/kg bw/bay as treatment-related and adverse as changes are >10% compared with controls during the intervals wk 1-4, wk 4-26, and wk 1-52/53.

Table 6.3.2-1: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Mean body weight and body weight changes [kg]

Dose level [mg/kg bw/day]	Males				Females			
	0	**30**	**125**	**500**	**0**	**30**	**125**	**500**
Mean bw prior to start (day -1)	8.2	8.3	8.3	8.3	7.4	7.4	7.6	7.4
Weeks 1 – 4	+0.6	+0.3	+0.5	**+0.2***	+0.3	+0.3	+0.3	+0.3
Weeks 4 – 26	+1.4	+0.9	+1.4	+1.1 (-21%)	+1.2	+1.1	+1.5	+1.6
Weeks 26 – 52	+0.9	+1.4	+1.1	+0.8 (-12%)	+0.6	+0.2	+0.5	+1.1
Weeks 1 – 52/53	+2.8	+2.6	+2.9	+2.0 (-29%)	+2.1	+1.6	+2.3	+3.0
Mean bw in week 52/53	11.2	11.0	11.2	10.5	9.6	9.2	10.0	10.6

* Statistically significant from control ($p < 0.05$)

The weight loss of some dogs observed in the control, and low-dose group during some periods of the study were resolved when the daily food quantity was increased. Therefore, these changes were considered not test substance related.

D. FOOD CONSUMPTION
There was no treatment-related effect on food consumption noted during the study.

The reduced food consumptions noted during the study were not considered test substance related, since they occurred only on some occasions and in control and treated dogs.

Due to weight loss one male each of the low and mid dose group, and one control female received 350 g/day from day 149, 180 and 185, respectively. From day 191 onwards all animals received 350 g/day. One male received 400 g from day 221 onwards.

E. OPHTHALMOLOGY

There were no ophthalmological findings observed at the end of the study period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related effects noted in the haematological parameters.

The differences observed for the activated partial thromboplastin time (week 25; males), MCHC (week 52; females) and eosinophil counts (week 52; females) in the treated animals when compared to control dogs were only slight and/or not dose-related.

Table 6.3.2-2: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Results of haematological examinations week 25 or week 51 (mean ± SD)

Parameter	Dose level [mg/kg bw/day]							
	Males				Females			
	0	30	125	500	0	30	125	500
Mean corpuscular haemoglobin concentration (MCHC) [g/dL] – week 51	35.4 ± 0.42	↓35.2 ± 0.84	↑35.5 ± 0.24	↓35.3 ± 0.19	36.0 ± 0.32	↓35.4 ± 0.56	↓35.5 ± 0.29	**↓35.0 ± 0.48***
Eosinophils [G/L] – week 51	0.27 ± 0.141	↑0.29 ± 0.087	↑0.33 ± 0.324	↑0.49 ± 0.275	0.37 ± 0.139	↓0.26 ± 0.127	↓0.30 ± 0.090	**↓0.19 ± 0.042***
Activated partial thromboplastin time [sec] – week 25	10.6 ± 0.55	10.4 ± 0.26	**9.8 ± 0.05***	10.6 ± 0.46	10.7 ± 0.69	10.2 ± 0.39	10.2 ± 0.61	10.7 ± 0.51

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Clinical chemistry

There were no treatment-related effects noted in the clinical chemistry parameters.
The differences observed for the inorganic phosphorous, calcium, protein, glucose, albumin/globulin ratio and AP values in the treated animals when compared to control dogs were only slight and/or not dose-related.

Table 6.3.2-3: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Results of clinical chemistry examinations at week 25 or week 51 (mean ± SD)

Parameter	Dose level [mg/kg bw/day]							
	Males				Females			
	0	30	125	500	0	30	125	500
Inorganic phosphorus [mmol/L] – week 25	1.28 ± 0.072	1.15 ± 0.063	1.21 ± 0.117	1.17 ± 0.078	1.29 ± 0.132	1.14 ± 0.055	**0.98 ± 0.161***	1.22 ± 0.136
Calcium [mmol/L] – week 25	2.82 ± 0.010	2.71 ± 0.099	2.79 ± 0.049	2.77 ± 0.80	2.78 ± 0.042	2.81 ± 0.139	2.80 ± 0.079	2.77 ± 0.040
Calcium [mmol/L] – week 50	2.80 ± 0.128	2.68 ± 0.125	2.75 ± 0.055	**2.63 ± 0.022***	2.75 ± 0.033	2.66 ± 0.085	2.77 ± 0.108	2.67 ± 0.092
Protein [g/L] – week 50	67 ± 4.3	64 ± 3.0	63 ± 2.2	**60 ± 1.9****	62 ± 2.2	60 ± 2.1	61 ± 2.1	64 ± 2.5

Table 6.3.2-3: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Results of clinical chemistry examinations at week 25 or week 51 (mean ± SD)

Parameter	Dose level [mg/kg bw/day]							
	Males				Females			
	0	30	125	500	0	30	125	500
Glucose [mmol/L] – week 25	5.53 ± 0.637	6.02 ± 0.795	5.99 ± 0.446	6.58 ± 0.449	4.98 ± 0.596	**6.22 ± 0.305***	5.91 ± 0.495	5.98 ± 0.050
Albumin/globulin ratio – week 50	1.21 ± 0.106	1.31 ± 0.090	1.30 ± 0.042	**1.39 ± 0.064***	1.40 ± 0.070	1.43 ± 0.048	1.38 ± 0.129	1.29 ± 0.088
Alkaline phosphatase [IU/L] – week 50	169 ± 79.5	167 ± 51.9	166 ± 47.9	119 ± 25.1	114 ± 29.7	212 ± 39.8	**230 ± 38.4***	177 ± 85.0

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINANALYSIS

There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

Organ weights

The statistically significant lower brain weight (see table below) observed in males at 125 mg/kg bw/day was dose-independent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.
There were no other statistically significant differences in organ weights and organ to body weight ratios between control and treated dogs.

Table 6.3.2-4: Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule) in Beagle Dogs [redacted] 2007): Body/Brain weights of male dogs

Dose group [mg/kg bw/day]	0	30	125	500
No of animals	4	4	4	4
Mean final body weight [g]	11165.0	10830.0	11090.0	10255.0
Mean brain weight [g]	87.41	80.06	**73.96****	84.09
Mean % of body weight	0.78978	0.74484	0.67578	0.82550

** DUNNETT'S TEST based on pooled variances at 1 % level; assigned control group(s): 1.

Gross pathology

There were no test substance related macroscopic findings observed in any animal of all dose groups.

Histopathology

There were no test substance related microscopic findings observed in any tissue sample of any dose group.

III. CONCLUSIONS

No unscheduled deaths or premature sacrifices occurred during the study. There were no treatment-related effects on clinical signs, eyes, body weight, body weight gain, food consumption, haematology, clinical chemistry or urine analysis parameters in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects.

The test item was clinically well tolerated and did not result in any laboratory or histological changes.
In conclusion under the experimental conditions of this study, the NOEL for oral toxicity of glyphosate technical was established at 500 mg/kg bw/day by the study authors.

Assessment and conclusion by applicant:

The toxicity potential of glyphosate technical was assessed in a 1-year oral toxicity study in male and female Beagle dogs. Groups of four dogs per sex received daily doses (capsules) of 0, 30, 125, or 500 mg/kg bw/day for 52 consecutive weeks. The study was conducted according to OECD 452 (1981) and in compliance with GLP regulations

Glyphosate technical was well tolerated and did not produce any laboratory or histological effects in Beagle dogs when administered daily for 52 weeks by the oral route at all dose levels. Based on these results, the NO(A)EL in Beagle dogs after 1 year of oral exposure was 500 mg/kg bw/day.

Assessment and conclusion by RMS:

The study is considered acceptable and the RMS agrees with the above assessment of the study. However, the RMS considers the reduced body weight gain in males at 500 mg/kg bw/bay (decrease by >10%, not-significant) as treatment-related and adverse. Therefore, the RMS proposes a NOAEL of 125 mg/kg bw/day based on these effects on body weight gain in males dosed at 500 mg/kg bw/day.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"This study is considered acceptable. It is agreed to set the NOAEL at the highest dose level of 500 mg/kg bw/day. It can be confirmed that the alterations in clinical chemistry parameters were mostly not statistically significant and, if so, did not show a dose response. The only possible exception is a lower blood calcium level in high dose males that was observed in other studies with glyphosate, too. However, without any concomitant findings, e.g, on bones, this perhaps treatment-related effects is not considered adverse.
This study was run in the same laboratory and under similar conditions as the 90-day study by [redacted] *(2007, ASB2012-11454) in which severe adverse effects were seen upon treatment of Beagle dogs with glyphosate at a high dose level of 1000 mg/kg bw/day. It is clear now that these adverse reaction to treatment was in fact confined to an exaggerated dose level and that the NOAEL is higher than 300 mg/kg bw/day as established in that previous study."*

B.6.3.2.23. Short term dog - one-year oral – study 2

Data point	CA 5.3.2/032
Report author	[redacted]
Report year	1997
Report title	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs
Report No	[redacted]94-0157
Document No	Not reported
Guidelines followed in study	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 409 (1981; in general compliance to OECD 452, 1981)
Deviations from current test guideline (OECD 409, 1998; OECD 452, 2018)	Blood clotting time parameters not evaluated; epididymis and uterus weights not reported. Deviations from the current versions of OECD 409 (1998) and OECD 452 (2018) are due to the fact that the study was aligned to older versions of the OECD test guidelines.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, category 2a **Conclusion AGG:** Acceptable.

Executive Summary

An oral 12-month toxicity study of HR-001 (Glyphosate technical) was conducted in Beagle dogs of both sexes. Groups of 4 males and 4 females each were given the test substance by incorporating it into basal diet at a level of 0, 1600, 8000 or 50000 ppm (equivalent to 0, 34.1, 182 or 1203 mg/kg bw/day for males and 0, 37.1, 184 or 1259 mg/kg bw/day for females) for a period of 12 months. Animals were checked daily for general conditions. Body weights and food consumption were measured periodically. All animals were subjected to urinalysis at weeks 25 and 51 and to haematology and blood chemistry at weeks 26 and 52. Ophthalmological examinations were performed at week 52. At termination of treatment, animals were euthanised and subjected to organ weight analysis and necropsy. Histopathological examinations were performed on representative organs/tissues from all animal used.

Findings related to the treatment were demonstrated in clinical observation, body weight, urinalysis, haematology and blood chemistry.

50000 ppm group: Loose stool was observed in 3 of 4 males and 4 of 4 females. These animals frequently showed this clinical sign through the treatment period, whereas in the control group, only one animal in each sex showed the sign over a limited period during treatment. Body weight gain was retarded gradually with progression of the treatment in both sexes, when compared to the controls. Consequently, the difference in mean body weight between the 50000 ppm and control groups became great with time, although statistical significance was not observed. Haematologically, slight anaemic changes were noted for females at weeks 26 and 52. Females also showed significantly increased plasma level of chloride at week 26 and significantly decreased plasma levels of albumin and inorganic phosphorous at week 52. Significantly lowered urine pH values were continuously observed in males and females. However, this finding was not recognised as a toxic change since it is known that the test substance is secreted with little metabolism into urine, is degraded to a free acid in urine, and consequently, makes the urine acidic. In the 50000 ppm group, an increased frequency in slight focal pneumonia was seen (all 4 females at the top dose vs 1 in the other dose groups and controls)

8000 and 1600 ppm groups: There were no treatment-related abnormalities in either sex.

A NOAEL of 8000 ppm (equivalent to 182 and 184 mg/kg bw/day for males and females, respectively) is proposed, which is based on loose stool in males and females, decreased body weight gain in males and females, decreased body weight in females at termination, slight anaemia in females, changes in blood electrolytes and an increased frequency of slight focal pneumonia in females observed at the top dose of 50000 ppm.

I. MATERIALS AND METHODS

A: Materials

1. Test material: Glyphosate technical

Identification:	HR-001
Description:	White crystals
Lot/Batch#:	T-950308
Purity:	94.61%
Stability of test compound:	Not mentioned in the report

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	5 months
Sex:	Males and females
Weight at dosing:	7.8 – 9.2 kg (males); 7.8 – 9.4 kg (females)

Acclimation period:	23 and 31 days for males and females, respectively
Diet/Food:	Solid diet DS (Oriental Yeast, Co.) restricted at 250 g/dog/day
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages 83.5 × 90.0 × 80.0 cm
Environmental conditions:	Temperature: 24 ± 2 °C Humidity: 55 ± 10 % Air changes: 15 / hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1996-03-05 to 1997-04-03

Animal assignment and treatment:
Groups of 4 males and 4 females Beagle dogs received the test material by incorporating it into the basal diet at a level of 0, 1600, 8000 or 50000 ppm for a period of 12 months.

Clinical observations
All animals were observed daily for clinical signs. Detailed clinical observations were performed at least once per week.

Body weight
Individual body weights were recorded at initiation of treatment, weekly from weeks 1 to 13, and every 4 weeks from weeks 16 to 52. In addition, final body weight was measured before necropsy.

Food consumption and compound intake
Food consumption of each animal was recorded weekly from week 1 to 13 and every 4 weeks from week 16 to 52. Food residues, if any, were collected and weighed every morning. Daily food consumption by each animal was calculated as follows:

$$Food\ consumption = \frac{[Feeding\ amount\ (250\ g\ diet + 250\ g\ water) - \ food\ residue]}{2}$$

Chemical intake (mg/kg bw/day) was calculated weekly from food consumption and body weight data and the nominal level.

Ophthalmological examination
Ophthalmological examinations were performed on all dogs prior to start of the treatment period and at week 52. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body and fundus.

Haematology and clinical chemistry
Blood samples were collected from all dogs prior to treatment, in weeks 25 and 52. The following haematological parameters were examined: Haematocrit, haemoglobin concentration, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count and total and differential leukocyte count.

All animals were subjected to blood biochemical examinations at weeks 26 and 52.

The following clinical chemistry parameters were examined: Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), γ-glutamyl transpeptidase (GGTP), creatine phosphokinase (CPK), creatinine (Creat.), blood urea nitrogen (BUN), total protein (TP), albumin (Alb), globulin (Glob.), albumin/globulin ratio (A/G ratio), glucose (Gluc.), total cholesterol (T. Chol.), triglyceride (TG), total bilirubin (T. Bil.), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K) and chloride (Cl).

Urinalysis

Prior to initiation of treatment and at weeks 25 and 51, all animals were subjected to urinalysis on the following parameters: Appearance, colour, specific gravity, pH, volume, proteins, glucose, ketones, bilirubin, nitrites, blood and urobilinogen.

Sacrifice and pathology
All surviving dogs were killed after completion of 52 weeks treatment and were subjected to a gross pathological examination. The following organs were weight: Adrenals, brain, epididymides, heart, kidneys, liver, spleen, thymus, uterus, pancreas, pituitary, prostate, ovaries, testes, thyroids with parathyroid. Organ to body weight ratios were calculated.

Tissue samples were taken from the following organs of all dogs and preserved in 10 % buffered formalin (except for the eyes with the optic nerve which were fixed in Davidson´s fixative, and testes and epididymides which were preserved in Bouin´s fluid): Brain (cerebrum, cerebellum, pons and medulla oblongata), spinal cord (cervical, thoracic and lumbar regions), peripheral nerve (sciatic nerve), pituitary, thymus, thyroids with parathyroids, adrenals, tonsil, spleen, bone with marrow (sternum and femur), lymph nodes (cervical and mesenteric), heart, aorta, tongue, buccal mucosa of oral cavity, pharynx, salivary glands (submaxillary and sublingual), oesophagus, stomach, liver, gallbladder, pancreas, duodenum, jejunum, ileum, caecum, colon, rectum, nasal cavity, larynx, trachea, lung, kidneys, urinary bladder, testes, prostate, penis, epididymides, ovaries, oviducts, uterus, vagina, diaphragm, eyes with optic nerve, femoral muscle, skin, mammary gland and all gross lesions.

A detailed histopathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

Statistics
The following statistical methods were used to test the significance of the differences:

Multiple comparison test - Dunnett's method: Body weight, Urine specific gravity, Urine volume, Haematology data, Blood biochemistry data, Organ weights

Mann-Whitney's U test: Food consumption, Urinalysis data (except urine volume and specific gravity)

Fisher's exact probability test: Mortality, Incidence of clinical signs and ophthalmological and pathological findings

In the multiple comparison test, data were first examined by Bartlett's test for homogeneity of variances among groups . When the variances were homogeneous, the standard one way classification analysis of variance was used to determine whether all the group means were homogeneous or not. When the group means proved tobe heterogeneous, Dunnett's multiple comparison test was applied. When it was shown by Bartlett's test that the variances were heterogeneous, the data were evaluated by Kruskal-Wallis test. If significant differences were indicated by this non-parametric procedure, Dunnett's type mean rank sum test was applied.

II. RESULTS AND DISCUSSION

Dietary analysis of test substance
The homogeneity of HR-001 in the 50000 ppm diet was examined using the samples taken from the top, middle, and bottom portions of the mixer at the first diet preparation. The result revealed the coefficient of variation on the concentrations of HR-001 in the 50000 ppm diet was 2.4%. Concentrations of HR-001 in test diets were monitored over a total of 14 times before and during the treatment. The overall mean concentrations (mean ± S.D.) found in the test diets of nominal levels of 1600, 8000, and 50000 ppm were 1534 ± 62.9, 7743 ± 483.9, and 48988 ± 2718.8 ppm, respectively, which corresponded to 96, 97, and 98% of the nominal levels (with a CV% of mean values of 4.1-6.3%).

A. MORTALITY
There were no deaths in any dose groups of either sex.

B. CLINICAL OBSERVATIONS
In the 50000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8000 and 1600 ppm groups did not show this clinical sign at all. In the control group, only one animal in each sex showed

it. Most of the animals in the 50000 ppm group frequently showed the sign throughout the treatment period, whereas the occurrence in the suffering animals of the control group was restricted to a limited period.

For other clinical signs observed, the occurrence was sporadic in all dose groups, or the incidence was almost comparable among the dose groups.

C. BODY WEIGHT

In the 50000 ppm group of both sexes, retarded body weight gain became evident gradually as the study progressed. Consequently, the mean body weights in this group at termination of treatment were 6% in males and 11% in females lower than those in the controls (males: 10.8 kg for top dose dogs vs. 11.5 kg in controls; females: 10.6 kg for top dose dogs vs. 11.9 kg in controls). However, statistically significant differences in mean body weights were not observed throughout the treatment between the control and treated groups including the 50000 ppm.

Table 6.3.2-1: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 1997): Mean body weight and body weight changes [kg]

Dose level [ppm]	Males				Females			
	0	1600	8000	50000	0	1600	8000	50000
Mean bw prior to start (day -1)	8.4	8.3	8.3	8.3	8.2	8.2	8.2	8.2
Weeks 0 – 4	+1.1	+1.2	+1.1	+0.9	+1.0	+0.9	+0.8	+0.6
Weeks 4 – 24	+2	+2.7	+1.8	+1.5	+1.9	+1.7	+2	+1.3
Weeks 24 – 52	0	+0.6	+0.5	+0.1	+0.8	+0.8	+1.4	+0.5
Weeks 0 – 52	+3.1	+4.5	+3.4	+2.5 (-19%)	+3.7	+3.4	+4.2	+2.4 (-35%)
Mean bw in week 52/53	11.5	12.8	11.7	10.8	11.9	11.6	12.4	10.6 (-11%)

* Statistically significant from control ($p < 0.05$)

D. FOOD CONSUMPTION

Decreased food consumption was noted for one female in the 1600 ppm group at weeks 24, 28 and 52 and for another female in the same group at week 32. Consequently, group mean food consumption in this group was decreased at those weeks. However, food consumption in this group recorded at other weeks was comparable to that of the controls. Moreover, the averaged group mean food consumption through the treatment period was almost comparable between the 1600 ppm and control groups of females.

All males in all dose groups and females except the above 2 animals in the 1600 ppm group consumed whole amount of diet offered every day.

Group mean chemical intakes were calculated from group mean values of food consumption and body weight, and the nominal dose levels. The overall group mean chemical intakes [mg/kg bw/day] through the whole treatment period are presented in the table below:

Table 6.3.2-2: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 1997): Mean test substance intake

Dose level [ppm]	Test substance intake [mg/kg bw/day]	
	Males	Females
1600	34.1	37.1
8000	182	184
50000	1203	1259

E. OPHTHALMOLOGY

No remarkable ocular changes were detected in animals in any dose group at week 52.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Statistically significant changes in haematology that were observed in treated groups are presented in the following table:

Table 6.3.2-3: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 1997): Results of haematological examination – selected parameters (mean ± SD)

Parameter	Week	Dose level [ppm]							
		Males				Females			
		0	1600	8000	50000	0	1600	8000	50000
Haematocrit (Ht) [%]	0	41.5 ± 1.7	40.9 ± 2.9	38.2 ± 1.4	39.5 ± 3.0	45.6 ± 0.6	42.1 ± 3.8	45.6 ± 1.8	**41.8* ± 1.2**
	26	48.2 ± 1.8	50.6 ± 2.1	48.4 ± 3.6	45.3 ± 2.8	49.1 ± 4.0	48.7 ± 1.6	45.8 ± 2.3	44.3 ± 1.9
	52	50.5 ± 2.0	53.8 ± 2.4	49.8 ± 3.3	47.6 ± 3.5	54.2 ± 3.7	53.1 ± 3.5	49.6 ± 4.3	**46.5* ± 3.1 (-14%)**
Haemoglobin (Hb) [g/dL]	0	14.2 ± 0.5	13.8 ± 1.1	13.0 ± 0.4	13.6 ± 1.0	15.1 ± 0.4	14.2 ± 1.2	15.5 ± 0.5	14.1 ± 0.4
	26	17.1 ± 0.7	17.5 ± 0.6	17.0 ± 1.0	16.0 ± 1.0	17.2 ± 1.2	17.1 ± 0.7	16.1 ± 0.6	**15.4* ± 0.5**
	52	17.3 ± 0.5	18.1 ± 0.7	16.8 ± 1.0	16.2 ± 1.1	18.2 ± 1.2	17.9 ± 1.0	16.8 ± 1.4	**15.7* ± 0.7 (-14%)**
Erythrocyte count (RBC) [$10^6/mm^3$]	0	6.31 ± 0.38	6.22 ± 0.70	5.68 ± 0.35	6.13 ± 0.69	7.08 ± 0.41	6.41 ± 0.50	6.94 ± 0.40	**6.26* ± 0.29**
	26	7.39 ± 0.41	7.83 ± 0.47	7.27 ± 0.62	7.09 ± 0.62	7.69 ± 0.47	7.37 ± 0.19	7.04 ± 0.53	6.94 ± 0.47
	52	7.68 ± 0.35	8.05 ± 0.63	7.31 ± 0.52	7.13 ± 0.71	8.40 ± 0.42	7.86 ± 0.62	7.46 ± 1.03	**6.87* ± 0.42 (-18%)**

* Statistically significant from controls ($p < 0.05$)

Male groups showed no significant changes in any parameters.

Female in the 50000 ppm group showed significantly decreased values of haematocrit (Ht), haemoglobin concentration (Hb), and erythrocyte count (RBC) at week 52. Haemoglobin concentration in this group was also significantly lower at week 26. This group had already showed lower values for these 3 parameters than the controls before initiation of treatment (at week 0). In particular, the differences from the control values in haematocrit and erythrocyte count at week 0 were statistically significant. However, the rates of deviation from the control values were, though slightly, augmented in the treatment period when compared to those at week 0.

Females in the 8000 and 1600 ppm groups showed no significant changes in haematological examinations.

Clinical chemistry

Statistically significant changes in blood biochemistry that were observed in treated groups are presented in the table hereafter:

Table 6.3.2-4: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 1997): Results of week 26 or week 52 clinical chemistry examination - selected parameters (mean ± SD)

Parameter	Dose level [ppm]							
	Males				Females			
	0	1600	8000	50000	0	1600	8000	50000
Creatine phosphokinase (CPK) [U/L] – week 52	111 ± 21	93 ± 7	**82* ± 8**	121 ± 16	78 ± 14	96 ± 9	103 ± 22	100 ± 20
Albumin (Alb) [g/dL] – week 52	3.07 ± 0.19	3.22 ± 0.18	2.99 ±0.26	2.88 ± 0.09	3.19 ± 0.14	3.04 ± 0.20	3.13 ± 0.12	**2.84* ± 0.17**

Table 6.3.2-4: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 1997): Results of week 26 or week 52 clinical chemistry examination- selected parameters (mean ± SD)

Parameter	Dose level [ppm]							
	Males				Females			
	0	1600	8000	50000	0	1600	8000	50000
Calcium (Ca) [mg/dL] – week 52	10.2 ± 0.2	10.4 ± 0.3	10.0 ± 0.3	9.8 ± 0.2	10.2 ± 0.1	**10.0* ± 0.1**	10.3 ± 0.2	**9.7** ± 0.1 (-5%)**
Inorganic phosphorus (P) [mg/dL] – week 52	3.8 ± 0.9	3.9 ± 0.6	3.3 ± 0.3	2.8 ± 0.2	3.9 ± 0.3	3.9 ± 0.3	4.2 ± 0.5	**2.8* ± 0.5 (-28%)**
Chloride (Cl) [mEq/L] – week 26	111.8 ± 1.4	111.7 ± 1.7	113.4 ± 0.9	113.9 ± 2.1	111.9 ± 0.9	112 9 ± 1.0	111.0 ± 1.9	**115.1* ± 2.1 (+3%)**
Chloride (Cl) [mEq/L] – week 52	112.3 ± 1.3	110.2 ± 1.8	112.5 ± 2.2	114.0 ± 1.0	112.0 ± 1.3	112.4 ± 1.5	109.3 ± 2.0	114.0 ± 1.7 (+2%)

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Females in the 50000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca) and inorganic phosphorous (P) at week 52. A significant decrease in calcium was also noted for females in the 1600 ppm group at 52 weeks.

For male groups, the 8000 ppm group showed a significant decrease in creatine phosphokinase (CPK) at week 52. But this change was not observed in the 50000 ppm group.

G. URINALYSIS

At 50000 ppm, there was a shift towards a lower urinary pH in both sexes at week 25 and week 51. There were no other findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

Organ weights

Males in the 1600 ppm group showed statistically significant increases in both absolute and relative weights of the pituitary. However, these changes were not observed in the 50000 or 8000 ppm groups of males.

In the 50000 or 8000 ppm groups, neither males nor females showed statistically significant changes in any organ weights.

The RMS adds that absolute and relative thyroid weights in top dose males were increased although not significantly. The increased thyroid weights can be attributed to two animals which both showed c-cell hyperplasia in the thyroid.

Table 6.3.2-5: HR-001: 12-Month Oral Chronic Toxicity Study in Dogs [redacted] 1997): organ weights (mean ± SD)

Parameter	Dose level [ppm]							
	Males				Females			
	0	1600	8000	50000	0	1600	8000	50000
Thyroid weight – absolute (mg)	940 ± 98	1031 ± 263	1029 ± 219	1278 ± 420 **(+36%)**	916 ± 151	785 ± 113	951 ± 200	1002 ± 129
Thyroid weight – relative (mg)	0.0082 ± 0.0008	0.0080 ± 0.0019	0.0087 ± 0.0011	0.0121 ± 0.0046	0.0079 ± 0.0019	0.0068 ± 0.008	0.0077 ± 0.0011	0.0095 ± 0.0007

Gross pathology

The macroscopic lesions observed in the present study were all sporadic in nature and there were no statistically significant differences in the incidence between the control and treated groups.

Histopathology
In the 50000 ppm group, focal pneumonia / focal granulomatous pneumonia in the lung was observed in all females. In the other female groups including the control group, the lesion was observed in only one of 4 animals each. However, the extent of the lesions was very focal and the degree of intensity was slight in all cases including those of the 50000 ppm group. Statistically, no significant differences between the control and dose groups were found in incidence of any histological lesions, including the pulmonary lesion.

III. CONCLUSIONS

Findings related to the treatment were demonstrated in clinical observation, body weight, urinalysis, haematology and blood chemistry.

50000 ppm group: Loose stool was observed in 3 of 4 males and 4 of 4 females. These animals frequently showed this clinical sign through the treatment period, whereas in the control group, only one animal in each sex showed the sign over a limited period during treatment. Body weight gain was retarded gradually with progression of the treatment in both sexes, when compared to the controls. Consequently, the difference in mean body weight between the 50000 ppm and control groups became great with time, although statistical significance was not observed. Haematologically, slight anaemic changes were noted for females at weeks 26 and 52. Females also showed significantly increased plasma level of chloride at week 26 and significantly decreased plasma levels of albumin and inorganic phosphorous at week 52. Significantly lowered urine pH values were continuously observed in males and females. However, this finding was not recognised as a toxic change since it is known that the test substance is secreted with little metabolism into urine, is degraded to a free acid in urine, and consequently, makes the urine acidic.

8000 and 1600 ppm groups: There were no treatment-related abnormalities in either sex.

Based on the results, the no-observable-effect level, minimum toxic level and sure toxic level of HR-001 to Beagle dogs under the conditions of the present study were determined as follow.

	Males	**Females**
No observable effect level	8000 ppm (182 mg/kg bw/day)	8000 ppm (184 mg/kg bw/day)
Minimum toxic level	50000 ppm (1203 mg/kg bw/day)	50000 ppm (1259 mg/kg bw/day)
Sure toxic level	≥50000 ppm (≥1203 mg/kg bw/day)	≥50000 ppm (≥1259 mg/kg bw/day)

Assessment and conclusion by applicant:
This oral 12-month toxicity study with glyphosate technical (HR-001) was conducted in Beagle dogs of both sexes. Groups of 4 males and 4 females each were given the test material via the diet at dose levels of 0, 1600, 8000 or 50000 ppm (equivalent to 0, 34.1, 182 or 1203 mg/kg bw/day for males and 0, 37.1, 184 or 1259 mg/kg bw/day for females) for a period of 12 months. The study was conducted according to OECD 409 (1981) and in general accordance with OECD 452 (1981).
Based on the study results the NOAEL in Beagle dogs after 1-year oral exposure to HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg bw/day for males and females, respectively).

Assessment and conclusion by RMS:
The study is considered acceptable and is in compliance with OECD 409 (1981) and in general accordance with OECD 452 (1981). The RMS agrees with the assessment by the applicant. The NOAEL of 8000 ppm (equivalent to 182 and 184 mg/kg bw/day for males and females, respectively) is agreed and is based on loose stool in males and females, decreased body weight gain in males and females, decreased body weight in females at termination, slight anaemia in females, changes in blood electrolytes in females and an increased frequency of slight focal pneumonia in females observed at the top dose of 50000 ppm. A lower urinary pH

was noted in both sexes, however, this was not considered adverse but attributed to the acidity of the test substance. In addition, a higher thyroid weight was noted in top dose males, which both showed c-cell hyperplasia in the thyroid. A NOAEL of 8000 ppm was also proposed in the previous assessment of the study.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
" The study is considered acceptable although there was an uncertainty about the dose levels that were actually tested. In the original study summary (p. 17), dose levels of 2000, 10000, and 30000 ppm are mentioned. According to a different information on the same page and in the following part of the report, dose levels were 1600, 8000, and 50000 ppm. It is assumed that the latter is correct but this error might provoke some doubts about the quality assurance system of the performing laboratory. The NOAEL (assumed to be 8000 ppm), however, is agreed with."

B.6.3.2.24. Short term dog - one-year oral – study 3

Data point	CA 5.3.2/033
Report author	[redacted]
Report year	1996 (Study report)
Report title	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs
Report No	[redacted] P/5079
Document No	Not reported
Guidelines followed in study	OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)
Deviations from current test guideline (OECD 452, 2018)	Organ weight of heart, spleen, ovaries and uterus was not determined. Deviations from the current version of OECD 452 (2018) are due to the fact that the study was aligned to an older version of the OECD test guideline 452.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point	CA 5.3.2/034
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs – Appendix (individual animal data)
Report No	[redacted] P/5079
Document No	Not reported
Acceptability/Reliability	**Conclusion GRG:** Valid, category 2a **Conclusion AGG:** Acceptable.

Executive Summary
In a toxicity study, groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 or 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 or 926.2 mg/kg bw/day for females) for a period of at least 1 year.

Clinical observations and veterinary examinations (including ophthalmoscopy) were made and body weights, food consumption and clinical pathology parameters were measured and at the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem*. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

Mild toxicity was evident at 30000 ppm glyphosate acid, as a slight reduction in body weight in females throughout the latter half of the study. This reduction was generally independent of any reduction in food consumption and

does not, therefore, reflect a palatability effect. There were no other toxicologically significant effects and the pathological no-effect level was 30000 ppm glyphosate acid.

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in body weight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg bw/day for males and 926 mg/kg bw/day for females.

A NOAEL of 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day) is proposed, which is based on the decreased body weights in top dose females during the course of and at the end of the study.

I. MATERIALS AND METHODS

A: Materials

1. Test material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.s.
CAS#:	Not reported
Stability of test compound:	Confirmed by the Sponsor
2. Vehicle and/ or positive control:	Diet / none
3. Test animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	20 – 29 weeks
Source	[redacted]
Housing	Housed by treatment group (sexes separately) in indoor pens. The pens had a sleeping platform with heated floor underneath and interlinking gates which enable the dogs to be separated for feeding and dosing
Acclimatisation period	4 – 5 weeks
Diet	Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 19 ± 2 °C Humidity: 40 – 70 % Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B: Study design and methods

In-life dates: 1995-04-11 to 1996-04-12

Animal assignment
In a chronic toxicity study, groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 and 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 and 926.2 mg/kg bw/day for females) for a period of at least 1 year. A randomisation procedure was used which resulted in the even distribution of dogs (16 males and 16 females) to treatment groups according to body weight ensuring that litter mates were in different groups. Each morning, male dogs received 400 g and female dogs received 350 g of their appropriate experimental diet.

Table 6.3.2-1: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] 1996): Study design

Test group	Dietary concentration [ppm]	Dose to animal (Males / Females) [mg/kg bw/day]	Males	Females
Control	0	0 / 0	1 – 4	5 – 8
Low	3000	90.9 / 92.1	9 – 12	13 – 16
Mid	15000	440.3 / 447.8	17 – 20	21 – 24
High	30000	906.5 / 926.2	25 – 28	29 – 32

Diet preparation and analysis
The experimental diets were made in 60 kg batches, by direct addition of glyphosate acid (allowing for purity) to ground Laboratory A diet, and mixed thoroughly. Water was then added to each batch and mixed prior to pelleting. The pellets were dried in the residual heat of an autoclave, allowed to cool and were then stored in bins at room temperature.

Samples from all dietary levels (including controls) were taken at approximately two-monthly intervals throughout the study and analysed quantitatively for glyphosate acid. The homogeneity of glyphosate acid in Lab diet A was determined by analysing samples from the low and high dose levels. The chemical stability of glyphosate acid in diet was determined over a period of up to 10 weeks (69 days) for these same diets.

Samples were extracted with water. Portions of the supernatant were diluted with water to give sample solution concentrations within the range of the calibration standards. These were derivatised using 9-fluorenylmethylchloroformate (FMOCCL) and analysed by High Performance Liquid Chromatography (HPLC).

Concentration analysis results: The mean achieved concentrations of glyphosate acid in analysed dietary preparation were typically within 12% of nominal concentration. The overall mean concentrations were within 9% of target.

Homogeneity results: The homogeneity of glyphosate acid in diet at concentrations of 3000 ppm and 30000 ppm for a batch size of 60 kg was determined and considered satisfactory; percentage deviations from the overall mean where within 11%.

Stability results: The chemical stability of glyphosate acid in experimental diets (determined at concentrations of 3000 ppm and 30000 ppm) when stored at room temperature, was shown to be satisfactory for 69 days. This covered the period of usage on the present study.

Observations
All dogs were observed at least three times daily for clinical behavioural abnormalities (at dosing, after dosing and at the end of the working day) and, on a weekly basis, they were given a thorough examination. Individual, daily assessments of gastro-intestinal findings were made for up to 5 hours post dosing: any subsequent assessments were made on a group basis. All dogs were also given a full clinical examination by a veterinarian pre-study, during weeks 13, 26, 39 and prior to termination. The examination included cardiac and pulmonary auscultation.

Body weight
All dogs were weighed weekly, before feeding, throughout the pre-study period, on day 1 and thereafter at weekly intervals until termination.

Food consumption and test substance intake
Food residues were recorded daily, approximately 4 hours after feeding and any residual food was discarded. These measurements were made for at least 2 weeks pre-study and throughout the treatment period.

Ophthalmoscopic examination
The eyes of all dogs were examined pre-study, during weeks 13, 26, 39 and prior to termination.

Haematology and clinical chemistry

Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing EDTA or trisodium citrate and the following parameters measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin, prothrombin time, blood cell morphology, mean cell haemoglobin concentration, platelet count, total white cell count, differential white cell count, red cell distribution width, prothrombin time, activated partial thromboplastin time and bone marrow smears (taken but not examined).

Clinical chemistry
Blood was collected from all dogs in weeks -1, 4, 13, 26 and prior to termination into tubes containing lithium heparin and the following parameters measured: Urea, creatinine, glucose, albumin, total protein, cholesterol, triglycerides, total bilirubin, creatine kinase activity, alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma-glutamyl transferase activity, calcium, phosphorus (as phosphate), sodium, potassium and chloride.

Urinalysis
Urine was collected by catheterisation, pre-experimentally, in week 26 and during the week prior to termination. The following parameters were measured and recorded on each urine sample: Volume, colour (if abnormal), specific gravity, pH, glucose, ketones, protein, bilirubin and blood.
In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.

Investigations *post mortem*
Macroscopic examination
All animals were killed by exsanguination under terminal anaesthesia induced by intravenous administration of sodium pentobarbitone and examined *post mortem*.

Organ weights
From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed: Adrenal glands, brain, epididymides, thyroid glands, kidneys, liver and testes.

The left and right components of paired organs were weighed separately.

Tissue submission
The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative: Gross lesions including masses, adrenal gland, aorta, brain (cerebrum, cerebellum and brainstem), bone marrow (sternum), caecum, colon, duodenum, epididymides, eyes, femur (including stifle joint, stored not examined), gall bladder, heart, ileum, jejunum, kidney, liver, lung, lymph node – prescapular, lymph node – mesenteric, mammary gland (females only), peripheral nerve (sciatic), oesophagus, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland (submandibular and parotid), spinal cord (cervical, thoracic, lumbar), skin, spleen, sternum, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, uterus, cervix and voluntary muscle.

Microscopic examination
All processed tissues were examined by light microscopy.

Statistics
All data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS (1989).
- Analysis of variance: Organ weights, food consumption
- Analysis of covariance: body weights (separate for males and females), relative organ weights (separate for males and females), haematology (sexes combined), clinical chemistry (sexes combined), urinary analysis (sexes combined).

II. RESULTS AND DISCUSSION

A. MORTALITY
None of the dogs died.

B. CLINICAL OBSERVATIONS

There were no toxicologically significant findings. Salivation at dosing was observed in individual dogs in all treatment groups throughout the study. The apparent increased incidence in two top dose males and one female was considered to be related to anticipation of feeding and not to treatment with glyphosate acid. There was also a low incidence of scrotal skin reddening seen in one male in each treatment group; this was considered to be incidental to treatment with glyphosate acid. There was no increased incidence of faecal abnormalities in dogs treated with glyphosate acid.

C. BODY WEIGHT AND BODY WEIGHT GAIN

There was a slight body weight effect evident in females fed 30000 ppm glyphosate acid with a maximum reduction of 11% (compared to controls) in week 51. These dogs showed a gradual reduction in growth rate, compared to the controls, which was consistently statistically significant from week 23 onwards. One female lost 0.6 kg during week 32 but this was related to a loss of appetite during this time. There were no effects in males at any dose level or in females at 15000 ppm but females fed 3000 ppm glyphosate acid also showed slightly poorer growth than the controls, with a maximum reduction of 8% in week 51. However, this effect only achieved statistical significance on occasions during the study and is considered attributable to the poorer growth of two females and not an effect of glyphosate acid, since there was no effect at 15000 ppm.

Table 6.3.2-2: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of body weights [kg] (selected time points; adjusted mean values shown for weeks 2-53)

Week	Dietary Concentration of Glyphosate acid [ppm]							
	Males				Females			
	0	3000	15000	30000	0	3000	15000	30000
1	11.40 ± 1.19	11.53 ± 0.83	11.33 ± 0.95	11.45 ± 0.83	9.60 ± 0.80	9.55 ± 0.69	9.48 ± 0.55	9.58 ± 0.99
8	12.66 ± 0.92	12.40 ± 0.49	12.48 ± 0.82	12.37 ± 0.88	10.74 ± 0.88	**10.40* ± 0.66**	10.68 ± 0.73	**10.42* ± 0.81**
16	13.35 ± 0.99	12.97 ± 0.92	13.28 ± 0.83	12.95 ± 0.79	11.46 ± 0.94	**11.03* ± 0.61**	11.50 ± 0.96	**10.99* ± 0.87**
32	14.19 ± 0.94	13.69 ± 1.48	13.93 ± 1.15	13.69 ± 0.85	12.28 ± 0.80	**11.63* ± 0.49**	12.59 ± 1.35	**11.46** ± 1.17**
53	14.57 ± 0.77	14.24 ± 1.72	14.24 ± 1.75	13.85 ± 0.96	13.10 ± 0.92	12.25 ± 0.51	12.94 ± 1.17	**11.76** ± 1.48 (-10%)**

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$ Student's t-test, 2-sided)

D. FOOD CONSUMPTION AND COMPOUND INTAKE

There was no effect on food consumption but 3 dogs (one male mid dose and 2 females top dose) left food on occasions which affected the group mean values. Dose rates (based on nominal dietary levels of glyphosate acid) were calculated in terms of mg/kg bw/day. Mean values are shown below:

Table 6.3.2-3: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] 1996): Mean Dose Received [mg/kg bw/day]

Glyphosate acid [ppm]	3000	15000	30000
Males	90.9	440.3	906.5
Females	92.1	447.8	926.2

E. OPHTHALMOSCOPIC EXAMINATION

There was a very low incidence of corneal or lenticular opacities but these were seen both in control animals as well as those fed glyphosate acid. There were no treatment related abnormalities.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology
There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry
There were no toxicologically significant findings.

Plasma cholesterol levels were increased slightly in the treated groups of both sexes at weeks 26 and 52 but there was no evidence of any dose relationship.

Plasma phosphorus levels were lower in the male treated groups at week 52 but this was due, in part, to slightly higher individual control values. ***The RMS notes that the same pattern was observed during week 4, 13, 26 and 52. The applicant is requested to provide HCD on phosphorus levels in blood in order to determine whether this was indeed due to higher (individual) control values.*** Similarly, the reduced sodium value in males fed 30000 ppm at week 52 was due solely to one male.

Various animals in all groups (including controls) showed evidence of higher plasma alanine aminotransferase, aspartate aminotransferase and creatine kinase activities throughout the study as well as pre-experimentally, but there was little evidence of any conclusive group effects.

Other statistically significant differences were minor and/or not dose related and were considered to be of no toxicological significance.

Table 6.3.2-4: Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] 1996): Intergroup comparison of blood clinical chemistry selected parameters and time points (adjusted mean values)

Parameter	Week	Dietary Concentration of Glyphosate acid [ppm]							
		Males				Females			
		0	3000	15000	30000	0	3000	15000	30000
Cholesterol [mmol/L]	26	3.78 ± 0.34	**4.48* ± 0.31**	3.96 ± 0.26	**4.40* ± 0.65**	4.28 ± 0.96	4.59 ± 0.70	**4.88* ± 0.60**	4.86 ± 0.36
	52	3.42 ± 0.10	**4.25* ± 0.42**	4.12 ± 0.39	**4.33* ± 0.58**	4.15 ± 0.97	4.32 ± 0.54	**5.08* ± 0.69**	**4.94* ± 0.61**
Phosphorus [mmol/L]	52	1.29 ± 0.23	**0.99* ± 0.15**	**0.89** ± 0.04**	**0.80** ± 0.16**	0.91 ± 0.16	1.12 ± 0.15	1.05 ± 0.07	0.76 ± 0.27
Sodium [mmol/L]	52	147.5 ± 1.5	146.2 ± 1.5	148.2 ± 1.3	**142.6* ± 6.9**	147.5 ± 1.0	147.3 ± 1.0	147.3 ± 1.7	148.1 ± 1.2

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS
There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.

H. SACRIFICE AND PATHOLOGY
Organ weights
There were no treatment-related effects on any organ weights. Adrenal weights were slightly raised in the male 3000 ppm group but this was exaggerated by a low value for one male in the control group.

Macroscopic findings
Several treated females showed red areas in or diffuse reddening of the urinary bladder mucosa. The incidence was not clearly related to dose and in the absence of a similar effect in males it was considered unlikely that the lesion is related to the administration of glyphosate acid.

Microscopic findings

It was considered unlikely that any of the lesions confined to the treated groups were related to the admin istration of glyphosate acid as they were either of low incidence or the incidence was not related to dose. The pathological no-effect level for glyphosate acid was 30000 ppm.

III. CONCLUSIONS

Mild toxicity was evident at 30000 ppm glyphosate acid, as a slight reduction in body weight in females throughout the latter half of the study. This reduction was generally independent of any reduction in food consumption and does not, therefore, reflect a palatability effect. There were no other toxicologically significant effects and the pathological no-effect level was 30000 ppm glyphosate acid.

Oral administration of 0, 3000, 15000 or 30000 ppm glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in body weight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg bw/day for males and 926 mg/kg bw/day for females.

There were no other treatment related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The no-observed adverse effect level (NOAEL) for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day). The no-observed adverse effect level (NOAEL) for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg bw/day).

Assessment and conclusion by applicant:
In this toxicity study, groups of four male and four female Beagle dogs were fed diets containing 0 (control), 3000, 15000 or 30000 ppm glyphosate acid (equivalent to 0, 90.9, 440.3 or 906.5 mg/kg bw/day for males and 0, 92.1, 447.8 or 926.2 mg/kg bw/day for females) for a period of at least 1 year. The study was conducted according to OECD 452 (1981) and in compliance with GLP (no certificate of the competent authority was provided).

Oral administration of glyphosate acid in the diet for 52 weeks caused minimal toxicity at 30000 ppm, evident as a slight reduction in body weight in females. This dose level was equivalent to an overall mean dose of 906 mg/kg bw/day for males and 926 mg/kg/ bw/day for females.
There were no other treatment-related findings and the pathological no-effect level was 30000 ppm glyphosate acid.

The NOAEL for toxicity over 1 year for females was 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day). The NOAEL for toxicity over 1 year for males was 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg bw/day).

Assessment and conclusion by RMS:

The study is considered acceptable and is in compliance with OECD 452 (1981). The RMS agrees with the assessment by the applicant. The NOAEL based on this study is 15000 ppm glyphosate acid (equivalent to an overall mean dose of 447 mg/kg bw/day). This is based on the decreased body weights in top dose females during the course of and at the end of the study.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"*The study is considered acceptable. Based on the reductions in body weight gain in high dose females, the NOAEL was the mid dose level of 15000 ppm, i.e., 447 mg/kg bw/day.* "

B.6.3.2.25. Short term dog - one-year oral – study 4

Data point	CA 5.3.2/035

Report author	[redacted]
Report year	1990
Report title	Glyphosate: 52 Week Oral Toxicity Study in Dogs
Report No	7502
Document No	Not reported
Guidelines followed in study	OECD 409 (1981), FIFRA 83-1, in general compliance with OECD 452 (1981)
Deviations from current test guideline (OECD 409, 1998; OECD 452, 2018)	Activated partial thromboplastin time not measured. Clinical signs poorly reported in the report. Deviations from the current versions of OECD 409 (1998) and OECD 452 (2018) are partial due to the fact that the study was aligned to older versions of the OECD test guidelines.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, category 2a **Conclusion AGG:** Acceptable.

Executive Summary
In a 52-week oral study, groups of four male and four female Beagle dogs were administered glyphosate daily via capsule at dose levels of 0, 30, 300 or 1000 mg/kg bw/day.

Clinical observations were made daily. Body weights and food consumption were assessed in weekly intervals. Ophthalmoscopic examination was performed pre-test and during weeks 13, 29, 39 and 51. Haematological, blood biochemistry parameters, as well as urine and faecal analysis for occult blood were conducted prior to start of treatment and at weeks 13, 26, 39 and 51 of treatment. Plasma levels of glyphosate were determined after weeks 1, 12 and 51 of treatment. Red blood cell and plasma cholinesterase activity were measured at weeks 13, 26, 39 and 51 and brain cholinesterase measured at termination. At the end of the scheduled period, the animals were sacrificed and subjected to a full examination *post mortem*. Selected organs were weighed and tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. Changes in faecal consistency (soft/loose/liquid) were recorded more frequently for the animals in the high dose group throughout the dosing period. This finding was observed 4 – 6 h after dosing and was also recorded on isolated occasions for a few animals of the intermediate dose group. A reduction in body weight gain was recorded for all treated groups males (low dose: approximately 17 % of control, intermediate and high dose: approximately 25 % of control). These body weight differences did not achieve statistical significance and may have been a chance effect. Considering the great differences in body weight gain between individual dogs within the treatment groups, the RMS agrees that the changes noted in males are considered to be due to this variability. In the females, only the high dose group showed a reduction in body weight gain of approximately 19 % of control. However, even when taking into account any outliers, the RMS considers the decreased body weight gain in high dose females as adverse and treatment-related.

The mean food consumption in treatment groups was not significantly different from controls. Ocular examinations revealed no treatment related abnormalities. There were no haematological or clinical chemistry changes observed that could be associated with treatment with glyphosate. Red blood cell, plasma, and brain cholinesterase activity was similar between all groups. There were no changes in any urinalysis parameters examined to indicate a treatment-related effect. Occult blood determinations performed on faecal samples obtained at the time of urine collections were negative for all animals throughout the study. There were no significant in absolute or relative (to body weight) organ weight differences between groups for either sex attributable to treatment with glyphosate. No pathologically significant gross or microscopic findings in any group could be related to treatment.

The RMS proposes a NOAEL of 300 mg/kg bw/day based on changes in faecal consistency (as also proposed by the applicant) but also on a decreased body weight gain in females at 1000 mg/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	Not reported
Lot/Batch#:	206-Jak-25-1; 206-Jak-59-5; 229-Jak-5-1;
Purity:	98.6 %; 99.5 %; 98.9 %
Stability of test compound:	Shown stable in capsule for at least 7 days

2. Vehicle and/or positive control: Clear gelatine capsule (empty)

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 5 – 6 months
Sex:	Male and female
Weight at dosing:	♂ 8.2 – 12.1 kg; ♀ 6.7 – 11.7 kg
Acclimation period:	3-weeks
Diet/Food:	SDS Dog Diet A, 400 g/day.
Water:	Tap water, *ad libitum*
Housing:	Paired in custom-designed pens
Environmental conditions:	Temperature: ~19 °C (12 – 25 °C) Humidity: ~50 % (30 – 83 %) Air changes: Not reported 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-08-29 to 1990-08-30

Animal assignment and treatment:
Four male and four female dogs per dose level received glyphosate technical (source [redacted]) orally, by capsule administration, once daily for 52 consecutive weeks. Three batches of the test material (206-Jak-25-1, purity: 98.6 %; 206-Jak-59-5, purity: 99.5 % and 229-Jak-5-1, purity: 98.9 %) were used during the course of the study. The bulk powder was encapsulated in hard, clear gelatin capsules. The individual test substance amount to be given was calculated weekly on the basis of each dog's most recently recorded body weight. Multiple-capsule administration was necessary in the high dose groups and, to ensure equal conditions, in the controls. The dose levels of 0 (vehicle control group receiving empty capsules), 30, 300 and 1000 mg/kg bw/day were selected on the basis of the results from a previous maximum tolerated dose study [redacted] 1989). Regular analyses of the capsule preparations performed at 3 monthly intervals revealed deviations of the actual from the nominal compound weight varying within tolerable limits (<5 %). Encapsulated Glyphosate was shown to be stable for at least 7 days in the capsules.

Table 6.3.2-1: Glyphosate: 52 Week Oral Toxicity Study in Dogs [redacted] 1990): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	4	4
Low	30	4	4

Table 6.3.2-1: Glyphosate: 52 Week Oral Toxicity Study in Dogs [redacted] 1990): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Intermediate	300	4	4
High	1000	4	4

Mortality
Each animal was checked for mortality or signs of morbidity during the daily clinical observations.

Clinical observations
A check for clinical signs of toxicity was made once daily.

Body weight
The body weight of each animal was recorded weekly starting two weeks before the start of treatment.

Food consumption and utilisation
Individual food consumption was recorded daily starting two weeks before the start of treatment.

Ophthalmoscopic examination
Ophthalmoscopy was performed at pre-test and again during weeks 13, 29, 39 and 51 of treatment.

Haematology and clinical chemistry
Laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and again during weeks 13, 26, 39 and 51 of treatment. The blood samples were taken from the jugular vein after the dogs had been fasted overnight, any food residues being withdrawn at approximately 1400 hours on the day before sampling.

EDTA was used as an anti-coagulant for evaluation of all parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time. Femoral bone marrow smears were taken at necropsy, air-dried and methanol-fixed but were not evaluated as there were no changes in peripheral blood or red cell morphology.

For clinical chemistry evaluations, heparin was used as an anti-coagulant and plasma analysed for the following parameters: Blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, sodium, potassium, calcium, chloride, creatinine, total protein, protein electrophoresis , albumin, albumin/globulin ratio, cholesterol, triglycerides, alkaline phosphatase, gamma glutamyl transpeptidase, phosphate, total bilirubin, red blood cell, plasma and brain cholinesterase.

Whole blood was collected from all the animals via the jugular vein into lithium heparinised tubes after l, 12 and 51 weeks of treatment. Immediately after collection, the samples were centrifuged, the plasma separated and stored deep frozen (-20 °C) until subsequent analysis for plasma levels of glyphosate.

Urinalysis/Faecal analysis
Urine samples were collected from all dogs housed in metabolism cages over the final 17 hours of a 21 hour period of water deprivation during weeks 13, 26, 39 and 51 of treatment. The following parameters were measured: Appearance, volume, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed for the presence of epithelial cells, white blood cells, red blood cells, crystals, organisms and abnormal constituents.
Faecal analysis for occult blood was performed at the time of the urine collections.

Sacrifice and pathology
After 52 weeks of consecutive treatment, all surviving animals were sacrificed by intravenous pentobarbitone followed by exsanguination and subjected to a gross pathological examination. Any macroscopic findings were recorded. Terminal body weights were recorded immediately after sacrifice.

The following organ weights were determined: Adrenals, thyroids with parathyroids, pituitary, brain, heart, liver and gallbladder (drained), kidneys, lungs, spleen, pancreas, thymus, testes with epididymides, prostate, uterus, ovaries and salivary gland (submaxillary, sublingual and parotid).

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aortic arch, brain (3 sections), caecum, colon, duodenum, epididymides, eyes with optic nerves (fixed in Davidson's fluid), heart, ileum, jejunum, kidneys, liver and gall bladder, lungs, lymph nodes (mandibular and mesenteric), mammary gland, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rectum, salivary gland (submaxillary, sublingual and parotid), spinal cord, sciatic nerve, skeletal muscle, skin, spleen, stomach, sternum, testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder and uterus. All tissues above from all groups were examined histopathologically. A sample of the brain was also stored for measurement of cholinesterase level.

Statistics
Haematology, clinical chemistry and body weight gain data were statistically analysed for homogeneity of variance using the 'F-max' test. If the group variance appeared homogeneous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variance were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances. If the variances remained heterogeneous then a non-parametric test such as Kruskal-Wallis ANOVA was used and pairwise comparisons made via the Dunn Z test where considered appropriate. Histological findings were analysed using Fisher's Exact Probability test. Organ weights were analysed relative to body weight.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Changes in faecal consistency (soft/loose/liquid) were recorded more frequently for the animals in the high dose group throughout the dosing period. This finding was observed 4 – 6 h after dosing and was also recorded on isolated occasions for a few animals of the intermediate dose group. There were no other clinical signs related to treatment with Glyphosate. Numerical incidence and frequency are not provided in the study report.

C. BODY WEIGHT
A lower body weight gain was recorded for males in all treated groups (low dose: approximately 17% of control, intermediate and high dose: approximately 25% of control). In the females, only the high dose group showed a lower body weight gain of approximately 19% of control. These differences did not achieve statistical significance and may have been a chance effect. The RMS partly agrees that this may have been a chance effect, mainly due high variation in individual body weight gain (refer to table below). There is one dog with a very high body weight gain in the male control group (7.4 kg, refer to table below). If this male would be excluded, the mean body weight gain value would be 3.8 kg in male controls and then the body weight gain effects among the treatment groups disappear. In addition, no clear dose-response was observed as mean body weight gain between 300 and 1000 mg/kg bw/day groups do not differ, which would have been expected if glyphosate would have an effect on body weight gain already at 30 mg/kg bw/day. On the other hand, the RMS considers the decreased body weight gain of 19% compared with controls in females treated at 1000 mg/kg bw/day as adverse and treatment-related. When considering the individual values, it is noted that one dog in the controls group has a quite low body weight gain (0.6 kg) and with excluding this dog the difference between controls and the female dogs treated at 1000 mg/kg bw/day will be even greater.

Table 6.3.2-2: Glyphosate: 52 Week Oral Toxicity Study in Dogs ([redacted] 1990): Intergroup comparison of mean body weights and body weight gain – selected time points from start of study

Dose [mg/kg bw/day]	Initial body weight [kg]	Final body weight [kg]	Total weight gain [kg]	Total weight gain [% of controls]
Males				
0	10.1	14.9	4.8 Individual values:	-

			3.9, 3.1, 7.4 and 4 5	
30	10.3	14.3	4.0 Individual values: 5.2, 4.2, 2.7 and 3.7	83 %
300	10.2	13.8	3.6 Individual values: 3.0, 1.9, 6.2 and 3.1	75 %
1000	10.3	13.9	3.6 Individual values: 4.2, 2.0, 4.6 and 3.3	75 %
Females				
0	8.8	12.4	3.6 Individual values: 4.3, 0.6, 3.4 and 5.9	-
30	8.7	12.6	3.9 Individual values: 4.0, 4.0, 2.0 and 4.8	108 %
300	9.0	13.1	4.1 Individual values: 4.5, 5.5, 3.8 and 2.8	114 %
1000	8.7	11.6	2.9 Individual values: 2.7, 2.6, 2.5 and 3.8	81 %

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

The mean food consumption in treatment groups was not significantly different from controls.

Analysis of glyphosate in the hard gelatin capsules showed an acceptable degree of accuracy in capsule preparation (<5 % deviation from nominal weight). There was no indication of degradation of encapsulated glyphosate over 7 days under the storage conditions employed. Plasma analysis indicated good proof of absorption and the levels of glyphosate in plasma were dose related. Plasma concentrations of glyphosate at week 1 ranged from 0.24 – 0 38, 1.29 – 2.17 and 5.44 – 10.23 µg/mL for low-, intermediate- and high dose males, respectively. Plasma concentrations of glyphosate at week 1 for females ranged from 0.16 – 1.01, 1.06 – 1.80 and 2.13 – 6.86 µg/mL for low-, intermediate- and high dose females, respectively. Plasma concentrations of glyphosate at week 52 ranged from 0.14 – 0.47, 0.99 – 2.64 and 3.18 – 9.49 µg/mL for low-, intermediate- and high dose males, respectively. Plasma concentrations of glyphosate at week 52 for females ranged from 0.24 – 0.63, 1.07 – 2.75 and 6.07 – 10.50 µg/mL for low-, intermediate- and high dose females, respectively.

E. OPHTHALMOSCOPIC EXAMINATION

Ocular examinations revealed no treatment-related abnormalities. There were incidental background observations which are commonly seen in dogs that were considered to be unrelated to treatment.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

All haematological parameters assessed gave values that were generally within the normal reference ranges for the species. There were no haematological changes observed that could be associated with treatment with glyphosate.

Blood clinical chemistry

There were no changes in the clinical chemistry parameters indicative of any treatment-related effects.

Red blood cell, plasma and brain cholinesterase activity was similar between all groups.

G. URINALYSIS AND FAECAL ANALYSIS

There were no changes in any of the parameters examined to indicate a treatment-related effect. Only the normal variations such as a few positive results for blood pigments and red blood cells were identified at pretrial and during the treatment period. In some instances, these positive results were attributable to the animals being in oestrous.

Occult blood determinations performed on faecal samples obtained at the time of urine collections were negative for all animals throughout the study.

H. NECROPSY

Organ weights

There were no significant absolute or relative (to body weight) organ weight differences between groups for either sex attributable to treatment with glyphosate.

Gross pathology

Very few findings were noted and there were no significant differences between controls and glyphosate -treated animals.

Histopathology

No pathologically significant findings were recorded in any group and there were no differences in incidences which could be related to treatment.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. Changes in faecal consistency (soft/loose/liquid) were recorded more frequently for the animals in the high dose group throughout the dosing period. This finding was observed 4 – 6 h after dosing and was also recorded on isolated occasions for a few animals of the intermediate dose group. A reduction in body weight gain was recorded for all treated groups males (low dose: approximately 17 % of control, intermediate and high dose: approximately 25 % of control). In the females, only the high dose group showed a reduction in body weight gain of approximately 19 % of control. These body weight differences did not achieve statistical significance and may have been a chance effect. The mean food consumption in treatment groups was not significantly different from controls. Ocular examinations revealed no treatment related abnormalities. There were no haematological or clinical chemistry changes observed that could be associated with treatment with glyphosate. Red blood cell, plasma, and brain cholinesterase activity was similar between all groups. There were no changes in any urinalysis parameters examined to indicate a treatment -related effect. Occult blood determinations performed on faecal samples obtained at the time of urine collections were negative for all animals throughout the study. There were no significant in absolute or relative (to body weight) organ weight differences between groups for either sex attributable to treatment with glyphosate. No pathologically significant gross or microscopic findings in any group could be related to treatment.

Chronic oral administration of glyphosate at dose levels up to 1000 mg/kg bw/day in Beagle dogs did not cause systemic organ toxicity. However, the 1000 mg/kg bw/day dose level (highest dose tested) was considered to be the maximum tolerated dose in view of the effect on faecal consistency at this dose level.

The no-effect level (NOEL) was judged to be 300 mg glyphosate/kg bw/day.

Assessment and conclusion by applicant:

In this 52-week oral study, groups of four male and four female Beagle dogs were administered glyphosate daily via capsule at dose levels of 0, 30, 300 or 1000 mg/kg bw/day. The study was conducted according to OECD 409 (1981) and in general compliance with OECD 452 (1981).

Chronic oral administration of glyphosate over a period of 52 weeks at dose levels up to 1000 mg/kg bw/day in Beagle dogs was free of systemic organ toxicity. However, the 1000 mg/kg bw/day highest dose level tested was considered to be the maximum tolerated dose in view of an effect on faecal consistency at this dose level. Therefore, the NOAEL was judged to be the intermediate dose of 300 mg/kg bw/day.

Assessment and conclusion by RMS:

The study is considered acceptable and is in compliance with OECD 409 (1981) and in general accordance with OECD 452 (1981). The RMS agrees with the assessment by the applicant and the NOAEL of 300 mg/kg

bw/day. However, the RMS considers this NOAEL based on changes in faecal consistency and on a decreased body weight gain in both sexes at 1000 mg/kg bw/day.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"The 12-month study of [redacted] (1990, TOX9552384) is still considered acceptable from a todays point of view and is included in the respective table in Volume 1 (2.6.3) and in this chapter. It is reported in detail in the old DAR (1998, ASB2010-10302).."

In the old DAR, the following was concluded:
" *The NOAEL in this study was 300 mg/kg bw/day based on changes in faecal consistency suggesting a rather common unspecific effect of treatment. A NOEL could not be established since a minor impact on body weight gain at least in male dogs cannot be excluded. The study author, in contrast, had argued that this decrease was due to random variation since statistical significance was not reached and assumed the NOEL at the mid dose level, therefore.* "

B.6.3.2.26. Short term dog - one-year oral – study 5

Data point	CA 5.3.2/036
Report author	[redacted]
Report year	1985
Report title	Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs
Report No	[redacted] 4965
Document No	Not reported
Guidelines followed in study	No guideline statement, but in general accordance with OECD 452 (1981)
Deviations from current test guideline (OECD 452, 2018)	Urine volume not measured, spleen and uterus not weighed, unclear number and location of brain sections observed microscopically. Deviations from the current version of OECD 452 (2018) are mainly due to the fact that the study was aligned to an older version of the OECD test guideline 452.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, category 2a **Conclusion AGG:** Acceptable.

Executive Summary
Glyphosate was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 20, 100 or 500 mg/kg bw/day for approximately twelve months.

Clinical observations were done twice daily. Weekly determinations of individual body weight were performed for fourteen weeks and bi-weekly determinations were made thereafter. Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals. Haematological, blood biochemistry parameters, as well as urine analysis, were conducted prior to start of treatment and during months 3, 6, and 12 of treatment. At the end of the scheduled dosing period, the animals were sacrificed and subjected to a full examination post mortem. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

There were no mortalities in any of the dose groups. Slightly higher incidences of abnormal excrement (bloody stool, yellow mucoid stool, diarrhoea, and emesis) were observed in low and high level females when compared to their control group. Approximately one-half of these observations were attributable to only one female dog from each of these groups of 6 animals per group. Females at the middle exposure level and males at all treatment levels had incidences of abnormal excrement similar to that of the control groups. One mid dose and one high dose female had localised skin redness with slight alopecia throughout most of the study. Body weight, food consumption and

ophthalmologic findings were normal throughout the test duration. Haematology, clinical chemistry and urine pathologic parameters plus gross and microscopic findings revealed no changes attributable to glyphosate exposure.

Since there was no conclusive evidence of toxicity in this study, the highest dose level of 500 mg/kg bw/day is considered the NOAEL.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White granular solid
Lot/Batch#:	NBP 2472136
Purity:	96.17 %
Stability of test compound:	Shown stable during the dosing period

2. Vehicle and/or positive control: Empty gelatin capsule

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	[redacted]
Age:	Approx. 6 months
Sex:	Male and female
Weight at dosing:	♂ 6.2 – 9.0 kg; ♀ 5.5 – 7.5 kg
Acclimation period:	Approx. 5 weeks
Diet/Food:	Purina Certified Dog Chow® 5007 (400 g available over 2 – 3 hour period)
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel dog cages
Environmental conditions:	Temperature: 20 – 22 °C (68 – 72 °F) Humidity: Not reported Air changes: Not reported

B: Study design and methods

In life dates: 1983-09-20 to 1984-09-24

Animal assignment and treatment:
Six male and six female dogs per dose level received glyphosate orally by gelatin capsule administration, once daily for approximately 12 consecutive months. Dogs were dosed approximately one to six hours after food was removed each day. Doses were adjusted to correspond with each animal's most recent body weight. Capsules were prepared each week. The dose levels were 0 (vehicle control group receiving one empty capsule), 20, 100 and 500 mg/kg bw/day. The high dose was set based on the maximum capsule size and number of capsules (two 1/8 oz. capsules) that could be reasonably administered daily to dogs of this size over one year. Each capsule could hold a maximum of approximately 3 grams of packed glyphosate.
Analyses after the completion of the study indicated 97.04 % glyphosate compared to an assay (conducted prior to the study) provided by the sponsor of 96.17 % glyphosate. The ± 0.87 % variation of analyses was within analytical limits and no decomposition of glyphosate was demonstrated.

Table 6.3.2-1: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs [redacted] 1985): Study design

Test group	Dose Level [mg/kg bw/day]	Males	Females
Control	0	6	6
Low	20	6	6
Mid	100	6	6
High	500	6	6

Mortality
Each animal was checked for mortality or signs of morbidity at least twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made at least twice daily (morning and afternoon) on all animals.

Body weight
Weekly determinations of individual body weight were performed for fourteen weeks. Bi-weekly determinations were made thereafter.

Food consumption and utilisation
Weekly determinations of daily food consumption were performed for fourteen weeks. Bi-weekly determinations were made thereafter.

Ophthalmoscopic examination
Ophthalmologic examinations were performed at pretest and prior to final necropsy on all animals.

Haematology and clinical chemistry
Laboratory investigations of haematology and clinical chemistry were performed on all the dogs before dosing started and again during months 3, 6, and 12 of treatment. The blood samples were taken from the jugular vein after the dogs had been fasted overnight.

EDTA was used as an anti-coagulant for evaluation of all parameters with the exception of prothrombin time for which citrate was used. The following haematological parameters were measured: Haemoglobin, haematocrit, red blood cell count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), reticulocyte count, total white blood cell and differential counts, platelet count and prothrombin time.

For clinical chemistry evaluations, serum was harvested from whole blood and analysed for the following parameters: Blood urea nitrogen, glucose, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, sodium, potassium, calcium, chloride, creatinine, total protein, albumin, globulin, cholesterol, alkaline phosphatase, gamma glutamyl transpeptidase, phosphorus, direct and total bilirubin.

Urinalysis
Urinalysis was performed on all the dogs before dosing started and again during months 3, 6, and 12 of treatment. Urine was collected using metabolism cages. The following parameters were measured: Appearance, pH, specific gravity, proteins, glucose, ketones, blood pigments, bilirubin and urobilinogen. Microscopic examination of the spun urine deposit was performed for the presence of bacteria, epithelial cells, white blood cells, red blood cells, crystals and abnormal constituents.

Sacrifice and pathology
After 12 months of consecutive treatment, all surviving animals were fasted overnight, sacrificed by intravenous sodium pentobarbital followed by exsanguination and subjected to a gross pathological examination. Terminal body weights were recorded immediately prior to sacrifice.

The following organ weights were determined: Adrenals, thyroids with parathyroids, pituitary, brain, heart, liver, kidneys, testes with epididymides and ovaries.

Tissue samples were taken from the following organs and preserved in buffered formalin: All gross lesions, adrenals, aorta, brain, caecum, colon, duodenum, epididymides, eyes (with optic nerves), heart, ileum, jejunum, kidneys, liver, gallbladder, lungs, lymph nodes (mesenteric), mammary gland, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rectum, rib, salivary gland (mandibular), spinal cord (cervical, mid-thoracic, lumbar), sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroids, trachea, ureter, urinary bladder and uterus. All tissues above from all groups were examined histopathologically.

Statistics
Non-categorical data from haematology, serum chemical and urinalysis were statistically examined by Dunnett's test for the comparison of multiple treatments with a control, and/or by inspection. Categorical data were examined to determine any remarkable group differences. Statistical evaluation of differences in body weights, food consumptions, terminal body weights and absolute organ weights between treated and control groups was accomplished by the use of Dunnett's test. The Mann-Whitney test with Bonferroni's inequality procedure was used to assess the organ/body weight ratios. Frequency of microscopic lesions between treated groups and controls was evaluated by the use of Fisher's Exact Test with Bonferroni's inequality procedure.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Slightly higher incidences of abnormal excrement (bloody stool, yellow mucoid stool, diarrhoea, emesis) were observed in low and high level females when compared to their control group. Approximately one half of these observations were attributable to one female dog (approx. 17 %) from each of these groups. Females at the middle exposure level and males at all treatment levels had incidences of abnormal excrement similar to that of the control groups. One mid dose and one high dose female had localized skin redness with slight alopecia throughout most of the study. One low dose male had similarly reddened skin, but his condition lasted for a relatively short time. All other animals' skin was unaffected. These findings were considered of questionable relationship to Glyphosate administration due to a lack of apparent dose-relationship. The remaining observations were not unusual and were not attributed to glyphosate exposure.

C. BODY WEIGHT
No significant body weight changes occurred between any of the dose groups.

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE
There were no treatment-related effects. Food consumption in treatment groups was comparable to controls. Test compound was administered by gelatin capsule with dosages adjusted according to individual animal body weight. Determination of the degree of absorption of the test item following dosing was note performed.

E. OPHTHALMOSCOPIC EXAMINATION
There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology
There were no changes in haematology parameters considered associated with glyphosate administration. Mild elevations in red blood cell counts (RBC), haemoglobin (HGB) and haematocrit (Hct) for low dose females at all test periods were similar to those observed at pretest. Mild changes in MCHC in males from the mid dose group (pretest and 12 months) and females from the low and mid dose groups (at 3 and 12 months) were not considered biologically significant.

Blood clinical chemistry
Phosphorus levels were statistically decreased in females from the low and high dose groups at 3 months and high dose group at 12 months when compared to controls. At 3 months, serum glutamic pyruvic transaminase (SGPT) values were significantly increased for high dose females. Sodium and potassium values were decreased in both sexes from high dose groups and for mid dose group males at 3 months, however this statistical difference was largely caused by slightly low control values at this time point. Statistically significant changes occurred in serum

sodium (mid dose), glucose (mid dose) and calcium (low dose) values among males at 12 months. At 6 months, increased albumin values were statistically significant in females from the low dose group.

Decreased phosphorus levels, although statistically significant in females at 3 and 12 months, did not appear to be related to compound administration since the values were within the normal range. ***The applicant is requested to provide HCD on phosphorus levels in blood in females.*** The changes in SGPT, sodium, potassium, calcium, glucose and albumin values were transient and/or within the normal range and were not considered an effect of glyphosate exposure.

Table 6.3.2-2: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs [redacted] 1985): Intergroup comparison of selected clinical chemistry parameters (group means)

Time point	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	20	100	500	0	20	100	500
Phosphorus [mg/dL)]								
Pre-test	6.5	6.4	6.4	6.6	6.6	6.3	6.7	6.0
3 months	5.4	5.3	5.1	5.1	5.2	**4.4***	4.5	**4.4***
6 months	3.8	4.0	4.3	3.9	4.1	3.8	3.8	3.7
12 months	3.2	3.4	3.3	2.8	3.8	3.5	3.5	**2.8***
SGPT [U/L]								
Pre-test	29.9	31.0	28.9	32.3	29.6	29.8	29.8	35.8
3 months	33.8	31.2	34.7	36.7	32.8	35.0	33.2	**42.8***
6 months	33.5	35.7	31.9	34.7	26.7	28.2	27.2	32.1
12 months	44.2	32.4	35.8	37.0	29.4	31.3	29.8	34.3
Sodium [mEq/L]								
Pre-test	146	147	146	146	144	145	146	144
3 months	150	149	**147****	**147****	151	150	149	**149***
6 months	147	149	148	149	142	142	141	142
12 months	148	149	**147***	148	147	147	148	147
Potassium [mEq/L]								
Pre-test	5.0	4.7	4.8	4.7	4.8	4.5	4.8	4.5
3 months	5.3	4.9	**4.7****	**4.5****	5.3	4.9	4.8	**4.5****
6 months	4.9	4.7	4.8	4.6	4.4	4.3	4.4	4.3
12 months	4.7	4.9	4.7	4.7	4.7	4.5	4.4	4.4
Calcium [mg/dL]								
Pre-test	11.6	11.5	11.5	11.5	11.2	11.3	11.2	11.2
3 months	10.8	11.0	10.7	10.8	11.1	11.1	11.0	11.1
6 months	10.6	10.4	10.5	10.6	10.5	10.8	10.5	10.5
12 months	10.4	**10.9***	10.2	10.1	10.8	11.2	10.8	10.7
Glucose [mg/dL]								
Pre-test	119	117	112	116	111	121	112	115
3 months	108	113	102	112	106	106	103	106
6 months	94.8	100	97.7	101	103	101	99.7	101
12 months	108	101	**96.0***	102	106	103	99.2	98.5

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

G. URINALYSIS

There were no urine abnormalities detected at any of the sampling periods.

H. NECROPSY

Organ weights

Absolute and relative (to body weight) pituitary weights for males from mid and high dose groups and absolute brain weights for males from mid dose group were statistically lower than controls at the terminal sacrifice. Reductions in pituitary weights were not conclusively associated with compound administration. There were no microscopic changes correlated with these reductions. Reduced brain weights did not follow a dose-related trend and were therefore not considered a treatment effect. No other significant differences were observed in absolute organ weights or organ to body weight ratios.

Table 6.3.2-3: Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs [redacted] 1985): Results from select absolute and relative organ weight determination

	Dose Level [mg/kg bw/day]							
	Males				Females			
	0	20	100	500	0	20	100	500
Mean pituitary weight [g]	0.084 ± 0.003	0.080 ± 0.003	**0.062** ** ± 0.003**	**0.068** ** ± 0.004**	0.059 ± 0.010	0.075 ± 0.004	0.068 ± 0.008	0.068 ± 0.004
Relative pituitary weight [% bw]	0.001 ± 0.000	0.001 ± 0.000	**0.001* ± 0.000**	**0.001* ± 0.000**	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000

* Statistically significant from controls ($p < 0.05$);
** Statistically significant from controls ($p < 0.01$)

Gross pathology
There were no compound-related or biologically significant differences in the incidence of gross lesions between the glyphosate treated animals and controls.

Histopathology
There were no histopathological findings related to treatment.

III. CONCLUSIONS

There were no mortalities in any of the dose groups. Slightly higher incidences of abnormal excrement (bloody stool, yellow mucoid stool, diarrhoea, and emesis) were observed in low and high level females when compared to their control group. Approximately one-half of these observations were attributable to only one female dog from each of these groups of 6 animals per group. Females at the middle exposure level and males at all treatment levels had incidences of abnormal excrement similar to that of the control groups. One mid dose and one high dose female had localised skin redness with slight alopecia throughout most of the study. Body weight, food consumption and ophthalmologic findings were normal throughout the test duration. Haematology, clinical chemistry and urine pathologic parameters plus gross and microscopic findings revealed no changes attributable to glyphosate exposure.

Since there was no conclusive evidence of toxicity in this study, the highest dosage (500 mg/kg bw/day) was considered a no-effect level (NOEL) by the study authors.

Assessment and conclusion by applicant:
In this study, glyphosate was administered orally by gelatin capsule to groups of six male and six female Beagle dogs at daily doses of 0, 20, 100 or 500 mg/kg bw/day for approximately twelve months. The study was conducted according to a testing regime in general accordance with OECD 452 (1981) and in compliance with GLP.

Although some unusual clinical observations (abnormal excrement, alopecia and skin redness) were noted among a few animals, these changes did not appear dose-dependent and were of questionable significance. Body weight, food consumption and ophthalmologic findings were normal throughout the test duration. All dogs survived the twelve months of testing. Clinical pathologic parameters plus gross and microscopic findings revealed no changes attributable to glyphosate exposure. Since there was no conclusive evidence of toxicity in this study, the highest dosage of 500 mg/kg bw/day is considered a no observed adverse effect level (NOAEL).

Assessment and conclusion by RMS:

The study is considered acceptable and is in general accordance with OECD 452 (1981). The RMS agrees with the assessment by the applicant and the NOAEL 500 mg/kg bw/day, the highest dose tested. This conclusion is in agreement with the previous assessment.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"… excluded from current re-evaluation on glyphosate, as well as a one-year study by [redacted] (1985, Z35385) that was briefly described in the 1998 DAR (ASB2010-10302) but had never been submitted as part of an EU dossier, neither in the 1990ies nor in 2012. It is available in Germany but, without effects up to the highest dose level of 500 mg/kg bw/day, this study would not alter overall assessment."

In the old DAR, the NO(A)EL was set at 500 mg/kg bw/day, the highest dose tested.

B.6.3.2.27. Short term dog - one-year oral – study 6

Data point:	CA 5.3.2/037
Report author	[redacted]
Report year	1982 (Revision/English version 1992)
Report title	12 month dietary toxicity study with glyphosate in dogs
Report No	8012
Document No	Not reported
Guidelines followed in study	Not known
GLP	Not known, GLP not compulsory when study was conducted
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Four Beagle dogs per sex and dose were fed diets for 12 months containing 0, 30, 100 or 300 ppm (equivalent to approx. 0, 0.75, 2.5 and 7.5 mg/kg bw/day based on application of diet conversion factor 40 for dogs published by Derelanko 2008) of glyphosate (purity and source not specified). Animals were observed for clinical signs. Body weights and food consumption were measured. Haematological and clinical chemistry evaluations were performed. The frequency of these observations and measurements are not known. All animals were subjected to gross pathological examination and histopathology. Organ weights were determined.
Short description of results:	There were no treatment-related clinical signs and no haematological, clinical chemistry, gross or histopathological changes with the possible exception of rounded hepatocytes and narrower sinusoids observed in the livers of some (2/4) high dose male dogs and mid (2/4) and high dose (3/4) females but not in the low dose and in the control groups. Since there was no further evidence of morphological or functional liver alterations, the reported findings while possibly treatment-related were not considered adverse effects. Thus, the NOAEL in this study was the highest dose of 300 ppm (approx. 8 mg/kg bw/day for the sexes combined).
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supplementary only due to reporting deficiencies (test substance purity missing, other deficiencies not specifically defined). The dates of the experimental work were not included in the report. RAR (2015): The study was considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Therefore and since the study report is not available to GRG, this study is not considered valid by GRG.

	Conclusion GRG: The study is considered unacceptable, category 4b. **Conclusion AGG**: The study report (an English translation of the original report) has been made available to AGG by BVL. The RMS has evaluated the study and agrees with the previous conclusion that the study is not considered unacceptable due to serious reporting deficiencies, e.g. the purity and manufacturer of the test substance is not reported. Further, concentration, homogeneity and stability of the test substance was not verified in the test diet and reporting tables of body weight, food consumption, haematology, clinical chemistry and organ weights are missing. Therefore, the study results reported above could not be verified by the RMS. As the study is not considered acceptable, no NOAEL is proposed. It is however noted that rounded hepatocytes and narrower sinusoids were observed in the livers of some (2/4) high dose male dogs and mid (2/4) and high dose (3/4) females, but not in the low dose and in the control groups. There was no further evidence of morphological or functional liver alterations and therefore the reported findings while possibly treatment-related were not considered adverse effects. These histopathological changes were also seen in the 3-month dog study from the same laboratory (8011, 1982) but were not seen in any other dog study with glyphosate obtained from other manufacturers.
Reasons why the study report is not available for submission	The notifier has no access to this study report. The former RMS (BVL) has made the study report available to the current RMS.

B.6.3.3. Other routes

B.6.3.3.1. Dermal repeated dose toxicity – study 1

Data point:	CA 5.3.3/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 21-Day Dermal Toxicity Study in Rats
Report No	[redacted]/P/4985
Document No	Not reported
Guidelines followed in study	OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Data point:	CA 5.3.3/002
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 21-Day Dermal Toxicity Study in Rats – Appendix (individual animal data)
Report No	[redacted] P/4985

Document No	Not reported
Guidelines followed in study	OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)
Acceptability/Reliability:	**Conclusion GRG**: Valid, Category 2a **Conclusion AGG**: Acceptable

Executive Summary

In a dermal toxicity study groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received 6-hour dermal applications of 0 (control), 250, 500 or 1000 mg glyphosate acid/kg bw/day. Glyphosate acid was prepared as a paste using deionised water as the control substance and vehicle. A total of 15 applications were made over a 21 day period (5 applications per week).

Clinical observations were made and body weights and food consumption were measured, and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no clinical signs of systemic toxicity at any dose level and no adverse compound related effects on body weight, food consumption, haematology, clinical chemistry or organ weights. There was no evidence of toxicity at histopathological examination.

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg bw/day.

I. MATERIALS AND METHODS

A: Materials

1. Test material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6 % w/w a.s.
CAS#:	Not reported
Stability of test compound:	Not reported
2. Vehicle and/ or positive control:	Deionised water / none
3. Test animals:	
Species	Rat
Strain	Alpk:APfSD
Age/weight at dosing	6 – 8.5 weeks / males 214 – 249 g, females 193 – 227 g
Source	[redacted]
Housing	Individually, in cages on multiple rat racks suitable for animals of this strain and weight range expected during the course of the study.
Acclimatisation period	At least 5 days
Diet	Diet (PCD) supplied by Special Diet Services Limited, Witham, Essex, UK, *ad libitum*
Water	Mains water, *ad libitum*
Environmental conditions	Temperature: 21 ± 2 °C Humidity: 40 – 70 % Air changes: At least 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B: Study design and methods

In-life dates: 1996-01-10 to 1996-02-01

Animal assignment
The study was divided into ten (randomised blocks), each containing one cage per treatment group. The animals were randomly allocated to groups as shown below:

Table 6.3.3-1: Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats [redacted] 1996): Study design

Test group	Dose level of glyphosate acid [mg/kg bw/day]	Males	Females
Control	0	5	5
Low	250	5	5
Mid	500	5	5
High	1000	5	5

Preparation and treatment of animal skin
Sixteen to twenty-four hours before application of the test substance, the hair was removed with a pair of veterinary clippers from an area, approximately 10 cm × 5 cm, on the dorso-lumbar region of each animal. The rats were dosed dermally and the amount applied was calculated for each animal according to its weight at the time of dosing. The paste covered by a gauze patch (approximately 7 cm × 7 cm × 4-ply) was applied to the shorn back of each animal and was kept in contact with the skin for approximately 6 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film (7 cm × 7 cm) and was held in position using adhesive bandage (25 cm × 7.5 cm). This was secured by two pieces of PVC tape (approximately 2.5 cm × 20 cm) wrapped around the animal. The control animals were treated in a similar manner except that deionised water only was used. The rats were dosed sequentially in group order at approximately the same time each day.

At the end of each 6-hour contact period, the dressings were carefully removed. The skin, at the site of application, was cleansed using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

A total of 15 six-hour applications were made during a period of 21 days. During this time there were three two-day periods when the animals were not dosed. Following each application there was an 18-hour 'rest' period during which the animals were fitted with plastic collars to prevent oral contamination.

Observations
Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. Detailed clinical observations were recorded daily and after decontamination. Cage-side observations were also made as soon as possible after dosing, and towards the end of the working day.

Body weight
The body weight of each rat was recorded daily, immediately prior to application of the test substance where applicable and prior to termination on day 22.

Food consumption and test substance intake
Food consumption was recorded continuously throughout the study for each rat and calculated as a weekly mean (g food/rat/day).

Haematology and clinical chemistry
Blood was collected at termination, by cardiac puncture and the following parameters were examined: Haemoglobin, haematocrit, red blood cell count, mean cell volume, mean cell haemoglobin, red cell distribution width, activated partial thromboplastin time, mean cell haemoglobin concentration, platelet count, total white cell count, differential white cell count, blood cell morphology and prothrombin time.

Clinical chemistry
Blood was collected at termination, by cardiac puncture and the following parameters were examined: Urea, creatinine, glucose, albumin, total protein, cholesterol, triglycerides, total bilirubin, creatine kinase activity,

alkaline phosphatase activity, aspartate aminotransferase activity, alanine aminotransferase activity, gamma-glutamyl transferase activity, calcium, phosphorus (as phosphate), sodium, potassium and chloride.

Investigations *post mortem*
Macroscopic examination
All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights
From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed: Adrenal glands, kidneys, liver and testes.
Paired organs were weighed together.

Tissue submission
The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative: Gross lesions including masses, testis*, kidney, liver, adrenal gland*, epididymis*, treated skin and untreated skin.
*: Tissues marked were stored and not examined microscopically

Microscopic examination
All selected tissues processed from the control and 1000 mg glyphosate acid/kg bw/day, together with macroscopic abnormalities from these groups, were examined by light microscopy.

Statistics
Haematology, clinical chemistry, organ weights and weekly food consumption were analysed using analysis of variance. Body weights, on initial (day 1) body weight, organ weights on final body weight were analysed using analysis of covariance. Analyses of variance and covariance allowed for the replicate structure of the study design and were carried out using the GLM procedure in SAS(1989). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. 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.

II. RESULTS AND DISCUSSION

Mortality
There were no mortalities.

Clinical observations
There were no significant signs of toxicity at any dose level of glyphosate acid. Generally the clinical findings observed were consistent with those commonly seen in dermal studies as a consequence of bandaging and were considered not to be related to treatment with glyphosate acid. One female dosed at 500 mg/kg bw/day had desquamation during the latter part of the study (day 12-22). This was not considered toxicologically relevant as it was considered an isolated finding as it was not reported in any of the other dose groups.

Body weight and weight gain
There were no effects due to treatment with glyphosate acid on body weight at any dose level.

Food consumption
There were no effects due to treatment with glyphosate acid on body weight at any dose level.

Haematology
A minimal statistically significant increase in haemoglobin levels was observed in females dosed at 1000 mg glyphosate acid/kg bw/day. A statistically significant decrease compared with control was seen in red cell distribution width in females dosed at 250 and 1000 mg glyphosate acid/kg bw/day. In the absence of any adverse effects on the red cell parameters and as no clear dose-response was observed, these minor changes are considered not to be of toxicological significance.

Table 6.3.3-2: Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats [redacted] 1996): Intergroup comparison of selected haematology parameters

Parameter	Dose level of glyphosate acid [mg/kg bw/day]							
	Males				Females			
	0	250	500	1000	0	250	500	1000
Haemoglobin [g/dL]	15.2 ± 0.4	15.3 ± 0.6	15.3 ± 0.3	15.0 ± 0.4	13.9 ± 0.6	13.7 ± 0.8	14.1 ± 0.6	**14.6* ± 0.3**
Red cell distribution width [%]	13.1 ± 0.9	12.9 ± 0.9	12.6 ± 0.3	13.4 ± 0.6	13.8 ± 1.2	**12.4** ± 0.8**	13.0 ± 0.4	**12.6* ± 0.7**

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided);
** Statistically significant difference from control group mean ($p < 0.01$; Student's t-test, 2-sided)

Blood clinical chemistry
Females dosed at 1000 mg glyphosate acid/kg bw/day showed a minimal, but statistically significant increase in plasma urea levels, but there were no differences seen in the plasma creatinine levels. This minimal change in urea was considered not to be of toxicological significance. A minimal but statistically significant decrease in plasma triglycerides was observed in males dosed at 500 mg glyphosate acid/kg bw/day and as this did not form part of a dose response relationship was considered not to be treatment related.

Table 6.3.3-3: Glyphosate Acid: 21 Day Dermal Toxicity Study in Rats [redacted] 1996): Intergroup comparison of selected clinical chemistry parameters (mean values)

Parameter	Dose level of glyphosate acid [mg/kg bw/day]							
	Males				Females			
	0	250	500	1000	0	250	500	1000
Plasma urea [mmol/L]	8.4 ± 0.5	8.2 ± 0.2	8.5 ± 0.2	8.1 ± 0.8	7.6 ± 0.5	7.7 ± 1.0	6.9 ± 0.5	**8.6* ± 1.1**
Plasma creatinine [µmol/L]	56.8 ± 2.2	57.2 ± 4.1	57.8 ± 4.1	54.4 ± 4.7	58.2 ± 2.3	58.6 ± 3.4	58.0 ± 6.6	58.2 ± 2.4
Plasma trigycerides [mmol/L]	1.27 ± 0.28	1.01 ± 0.17	**0.87* ± 0.22**	1.27 ± 0.34	0.70 ± 0.11	0.66 ± 0.21	0.69 ± 0.19	0.76 ± 0.35

* Statistically significant difference from control group mean ($p < 0.05$; Student's t-test, 2-sided)

Sacrifice and pathology
Organ weights
Testes weights were slightly, but statistically significantly decreased at 500 mg glyphosate acid/kg bw/day (2.85 ± 0.32 g vs. 3.16 ± 0.18 g in controls), due to one animal having a relatively low weight recorded. As no effect was seen at the top dose and only one animal has a decreased testes weight, this finding is considered incidental. There were no effects due to treatment with glyphosate acid in the other organs weighed.

Macroscopic findings
A small number of lesions were observed in a few animals in the 0 and 500 mg/kg bw/day groups, none of which were related to treatment.

Microscopic findings
A small number of common spontaneous lesions were observed, none of which were related to treatment.

III. CONCLUSIONS

There were no clinical signs of systemic toxicity at any dose level and no adverse compound related effects on body weight, food consumption, haematology, clinical chemistry or organ weights. There was no evidence of toxicity at histopathological examination.

There was no evidence of systemic toxicity or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg bw/day.

The no-effect level (NOEL) for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg bw/day in both sexes.

Assessment and conclusion by applicant:
In this dermal toxicity study, groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received 6 hour dermal applications of 0 (control), 250, 500 or 1000 mg glyphosate acid/kg bw/day. The study was conducted according to OECD 410 (1981) and in compliance with GLP.
There was no evidence of systemic effects of toxicological significance or dermal irritation following 15 dermal applications over a 21 day period of up to 1000 mg glyphosate acid/kg bw/day.
The NOAEL for systemic toxicity and dermal irritation was considered to be 1000 mg glyphosate acid/kg bw/day in both sexes.

Assessment and conclusion by RMS:
The study is considered acceptable and the assessment by the applicant is agreed including the proposed NOAEL of 1000 mg/kg bw/day for both systemic toxicity and dermal irritation. This conclusion is in agreement with the previous assessment.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"*The study is considered acceptable even though most dermal studies in rats include a treatment period of four weeks while rabbits are administered a test substance via the skin usually for 21 days. We agree with the conclusions including setting of the NOAEL for both systemic effects and dermal irritation at the limit dose of 1000 mg/kg bw.*"

B.6.3.3.2. Dermal repeated dose toxicity – study 2

Data point:	CA 5.3.3/003
Report author	[redacted]
Report year	1993
Report title	Glyphosate: 3 Week Toxicity Study in Rats with Dermal Administration
Report No	7839
Document No	Not reported
Guidelines followed in study	US EPA 82-2 (Pesticide Assessment Guidelines, Subdivision F); in general compliance with OECD 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	Mean weight of the female rats were slightly lighter than requested (195 g instead of 200 – 300 g), organ weights of the adrenals were not determined.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG**: Valid, Category 2a **Conclusion AGG**: Acceptable

Executive Summary
A group of 5 male and 5 female Sprague-Dawley rats were dosed daily with glyphosate via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The group was dosed at a constant volume of 3 mL/kg body

weight at a dose level of 1000 mg glyphosate/kg bw/day. A further group of 5 males and 5 females received vehicle only (diethylphthalate) dermally at the same dose volume to act as a control. Blood samples were collected for haematology and clinical chemistry screens during week 3. After 3 weeks of dosing all rats were killed and necropsied and selected organs weighed. All control and high dose rats underwent a limited histological examination.

No effect on mortality or clinical signs could be observed. Findings in body weight changes or food/water consumption were not consistent and considered to be not treatment-related. The skin assessment showed a minor reaction to the treatment of glyphosate. Haematology, clinical chemistry or organ weights revealed no notable intergroup differences in either sex. Histopathological examination did not reveal any finding that could be attributed to dosing with glyphosate.

In conclusion, following dermal administration of glyphosate to Sprague-Dawley rats for 3 weeks at a dose level of 1000 mg/kg bw/day, there was no evidence of systemic toxicity. This dose level is considered the NOAEL for systemic effects. A mild transitory irritant effect was noted at the dosing site at 1000 mg/kg bw/day as this was the only dose level, no NOAEL for local effects could be derived. The dose level of 1000 mg/kg bw/day is considered the LOAEL for local effects as mild skin irritation (erythema and desquamation) was noted in animals following dermal administration of glyphosate at this dose level.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White crystalline solid
Lot/Batch#:	229-Jak-142-6
Purity:	101.5 %
Stability of test compound:	The article is stable at least 2 years from date of analysis when stored at ambient temperature in the dark

2. Vehicle and/or positive control: Diethylphthalate / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Ca. 8 weeks
Sex:	Male and female
Weight at dosing:	♂ 295 g; ♀ 195 g
Acclimation period:	25 days
Diet/Food:	SDS Rat and Mouse (modified) No. 1 Diet SQC (supplied by Special Diets Services Limited, Witham, Essex, CM8 3AD), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in polypropylene cages (overall dimensions ca 420 × 270 × 200 mm) with stainless steel wire grid tops and bottoms.
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 50 ± 15 % Air changes: 15 air changes per hour 12 hours light/dark cycle

B: Study design and methods

In life dates: 1992-04-06 to 1992-04-27

Animal assignment and treatment:
A group of 5 male and 5 female Sprague-Dawley rats were dosed daily with glyphosate via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The group was dosed at a constant volume of 3 mL/kg body weight at a dose level of 1000 mg/kg bw/day (high limit dose). A further group of 5 males and 5 females received vehicle only (diethylphthalate) dermally at the same dose volume to act as a Control.

Table 6.3.3-1: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Study design

Test group	Treatment [mg/kg bw/day]	Males	Females
Control (1)	0	5	5
High (2)	1000	5	5

The hair on the animals' backs was clipped as necessary (once during Week 1, twice during Week 3 and not clipped at all during Week 2) and the test material applied daily at the constant volume of 3 mL/kg bw, over an area of approximately 10 % of the total body surface area. After application of the test material the dosing site was covered by a piece of gauze with a silver foil back (approximately 4 cm diameter). The treated area was protected by a semi-occlusive dressing (Micropore, 3M) held in place by means of a non-irritating tape (Blenderm, 3M). Dermal exposure was ca 6 h per day, the site of exposure being cleaned of test material with a soft cloth soaked in diethylphthalate immediately after removal of the dressing. If the animals managed to remove the dressing during the dosing period it was re-applied for the remainder of the 6 h period. A record of animals which managed to remove the dressing was maintained and is shown below.

Table 6.3.3-2: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Dress removal of tested animals

Timepoint	Group/dose level [mg/kg bw/day]							
	Animals found without dressing				Dressing chewed			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
Week 1	0	0	No. 14 on one occasions	0	0	0	0	0
Week 2	0	0	No. 13 on two occasions No. 14 on five occasion	0	0	0	No. 14 on one occasion	0
Week 3	0	0	No. 14 on one occasion	0	0	0	No. 13 on one occasion No. 14 on one occasion	0

Preparation of Dosing Suspensions
Dosing suspensions were prepared daily, using diethylphthalate (DEP) as vehicle. Water was originally intended as the vehicle for the study. However, this formed a very poor suspension with glyphosate and allowed a constant dose volume of only 2 mL/kg bw. Therefore, after experimentation at [redacted], the vehicle was changed to diethylphthalate. This has been used successfully at [redacted] as a vehicle in previous dermal studies; it formed a far more homogeneous suspension with glyphosate, and allowed a constant dose volume of 3 mL/kg bw.

Analysis of the dosing suspensions

Analysis of the dosing suspension was conducted in another study and not reported.

Mortality
Animals were checked twice daily for mortality.

Clinical observations
All animals were examined for reaction to treatment during the day. The onset, intensity and duration of all signs were recorded. In addition, all animals received a detailed clinical examination once each week.

Skin Assessment
Once each week all animals received a detailed examination of skin for erythema, eschar formation and oedema formation.

Table 6.3.3-3: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Grading system for the skin assessment

Erythema and eschar formation	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Oedema formation	No oedema	0
	Very slight oedema (barely perceptible)	1
	Slight oedema (edges of area well defined by definite raising)	2
	Moderate oedema (edges raised approximately 1 mm)	3
	Severe oedema (raised by more than 1 mm and extending beyond the area of exposure)	4
Skin thickening	Normal	0
	Thicker than normal	1
Desquamation	No desquamation	0
	Mild desquamation (dry skin)	1
	Moderate desquamation (flaky skin)	2
	Severe desquamation (skin cracking)	3

Body weight
The weight of each animal was recorded twice during the week before the start of treatment, on five occasions during Week 1 and twice each week thereafter.

Food and water consumption
The quantity of food consumed by each animal was recorded twice each week starting 1 week pre-trial up until the end of the study. Water consumption was monitored by visual inspection throughout the treatment period.

Haematology and clinical chemistry
Samples were taken from all animals during Week 3. Blood samples were collected from the orbital sinus under light ether anaesthesia (except Hepato Quick which was taken by tail snip) with overnight food deprivation.

Haematology:
The following parameters were determined: Haematocrit, haemoglobin, total red blood cell count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, Hepato Quick (clotting time), total white blood cell count and differential white blood cell count.

Clinical chemistry
The following parameters were determined: Aspartate amino transferase (AST), alanine aminotransferase (ALT), creatinine (Crea), total protein (TP), albumin (Alb), AG ratio (AG-R), blood urea nitrogen (BUN), glucose (Glu), total bilirubin (T Bi), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphorus (P).

Sacrifice and pathology

All animals were killed and necropsied after 3 weeks dosing. Method of killing was by carbon dioxide asphyxiation followed by exsanguination. The gross dissection and necropsy were performed under the supervision of a pathologist.

Organ weights
The following organs were weighed: Kidneys, liver and testes with epididymides.

Histopathology
Histopathological examinations of the following organs were performed: Abnormal tissue, dosing site, kidneys, liver, lungs, normal skin, ovaries, spleen and testes with epididymides.

Statistics
Body weight, haematology and clinical chemistry 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 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 in an attempt to stabilise the variances. If the variances remained heterogeneous, then a non-parametric test such as Kruskal-Wallis ANOVA was used. Individual between group comparisons were made using Fisher's F-protected LSD method. Organ weights were analysed as above and conditional on body weight (i.e. analysis of covariance). Histology data were analysed using Fisher's Exact Probability test.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF THE FORMULATED DOSING SUSPENSIONS
Analysis of the formulated dosing suspension was conducted in another study and not reported.

B. MORTALITY
There were no premature deaths.

C. CLINICAL OBSERVATIONS
There were no clinical signs observed which were considered to be related to treatment with glyphosate.

D. SKIN ASSESSMENT
During Week 2, 2/5 male animals of the high dose group and 3/5 female animals of the high dose group showed very slight erythema, however, during Week 3, this finding was only apparent in 1/5 of the high dose females.

Also during Week 2, desquamation was apparent in 3/5 male animals of the high dose group (ranging from moderate to severe) and in all female high dose animals (ranging from mild to severe), however, during Week 3, only mild desquamation was apparent in 1/5 high dose males and thickening and severe desquamation were apparent in only 1/5 high dose females.

No findings were noted in control males or females at any time during the study.

Table 6.3.3-4: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration ([redacted] 1993): Skin assessment

Group / Dose level [mg/kg bw/day]							
Group 1/0 ♂		Group 1/0 ♀		Group 2/1000 ♂		Group 2/1000 ♀	
Animal #	Assessment	Animal #	Assessment	Animal #	Assessment	Animal #	Assessment
1	No reaction	11	No reaction	6	No reaction	16	Week 2: severe desquamation
2	No reaction	12	No reaction	7	Week 2: slight erythema and moderate desquamation	17	Week 2: slight erythema and moderate desquamation

Table 6.3.3-4: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Skin assessment

Group / Dose level [mg/kg bw/day]							
Group 1/0 ♂		**Group 1/0 ♀**		**Group 2/1000 ♂**		**Group 2/1000 ♀**	
Animal #	**Assessment**	**Animal #**	**Assessment**	**Animal #**	**Assessment**	**Animal #**	**Assessment**
3	No reaction	13	No reaction	8	Week 2: slight erythema and severe desquamation	18	Week 2: slight erythema and severe desquamation Week 3: slight erythema, thickened skin and severe desquamation
4	No reaction	14	No reaction	9	No reaction	19	Week 2: slight erythema and moderate desquamation
5	No reaction	15	No reaction	10	Week 2: moderate desquamation Week 3: mild desquamation	20	Week 2: mild desquamation

E. BODY WEIGHT

The high dose males showed a moderate reduction in body weight gain (17%) and the high dose females showed a large increase in body weight gain (58%) when compared to their respective controls.

In absolute terms, these changes did not reach statistical significance at all in males, and only once in females (during week 3, $p \leq 0.05$). This lack of statistical difference along with the fact that there was such a wide variation between the sexes and that similar changes have not been observed in previous studies using glyphosate led to the suggestion by the study authors that these changes are not related to treatment with glyphosate. RMS: as the decreased body weight gain in males is mainly due to one animal which had a much lower body weight gain (23 gram) in comparison to the other top dose males (54-80 gram), and as only five animals per group are used in this type of study making it more difficult to interpret any changes, this effect is not considered adverse.

There were no other notable intergroup differences.

Table 6.3.3-5: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Group Mean Body weight data [g]

Treatment period [days]	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	**2/1000 ♂**	**1/0 ♀**	**2/1000 ♀**
-7	241	249	172	172
-4	258	265	181	185
0	294	295	193	200
3	307	304	198	205
7	326	322	206	222
10	338	331	216	229
14	354	348	224	243
17	363	354	229	255
21	364	353	231	**260***
Body weight gain from day 0 – 21 [g]	70	58	38	60

Table 6.3.3-5: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Group Mean Body weight data [g]

Treatment period [days]	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
% of control	-	83	-	158

* Significantly different from control, $p \leq 0.05$

F. FOOD AND WATER CONSUMPTION

There were no notable intergroup differences for food consumption in males. In females, there was a slight increase in food consumption in the high dose females (14%) when compared to controls. With the lack of a similar effect in males, and as this finding has not been seen in previous studies using glyphosate, this increase was considered by the study authors a chance effect and not of toxicological importance. There were no other notable intergroup differences.

Table 6.3.3-6: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Summary of food consumption data [g/rat/time period]

Treatment period [days]	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀
-7 to -4	28.9	31.2	23.1	25.2
-4 to 0	32.1	31.8	22.3	25.5
0-3	33.5	31.1	21.9	25.1
3-7	35.5	33.2	23.5	26.8
7-10	33.3	32.9	24.5	28.0
10-14	34.8	33.7	25.9	30.5
14-17	34.7	34.0	26.5	29.5
17-21	38.3	39.2	31.1	34.7
Total food consumed from day 0 – 21 [g]	701	679	510	581
% of control	-	97	-	114

There were no visual intergroup differences in either sex noted for water consumption.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Males: There was a decrease in monocytes (44%, $p \leq 0.05$) and large unstained cells (62%, $p \leq 0.05$) in the high dose males compared to Controls.

The study authors stated that these findings have not been seen in previous studies using glyphosate and the decrease in monocytes may partly be due to higher than normal values in Control animals ([redacted] historical data show a mean of 0.226 ± 0.126, number of samples being 74). In addition, the RMS adds that as changes of monocytes and large unstained cells are within 3-fold of controls values, these changes are not considered adverse according to the JMPR guidance (2015).

There were no other notable intergroup differences.

Females: There was an increase in MCH (4%, $p \leq 0.05$) and MCV (4%, $p \leq 0.01$) in the high dose females compared to Controls. There was a reduction in neutrophils (31%, $p \leq 0.05$) in the high dose females compared to Controls. Again according to the study authors, these findings have not been seen in previous studies using glyphosate. There were no other notable intergroup differences.

Table 6.3.3-7: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Summary of selected group mean haematology data

Parameter	Group/Dose level [mg/kg bw/day]			
	1/0 ♂	2/1000 ♂	1/0 ♀	2/1000 ♀

	n = 5	n = 4 [a]	n = 5	n = 5
Monocytes [×10^9/L]	0.57 ±0.17	**0.32* ± 0.07**	0.23 ± 0.09	0.22 ± 0.11
Large unstained cells [×10^9/L]	0.29 ± 0.14	**0.11* ± 0.02**	0.06 ± 0.01	0.08 ± 0.04
Mean cell haemoglobin [pg]	19.6 ± 1.0	19.4 ± 0.4	19.9 ± 0.2	**20.7* ± 0.6**
Mean cell volume [fL]	54.6 ± 1.9	54.0 ± 1.3	53.5 ± 0.9	**55.8** ± 1.3**
Neutrophils [×10^9/L]	3.34 ± 1.65	1.99 ± 0.62	1.68 ± 0.30	**1.16* ± 0.41**

* Significantly different from Control, $p \leq 0.05$;
** Significantly different from Control, $p \leq 0.01$
[a] the blood sample from one animal clotted and no analysis of blood chemistry was possible.

Blood clinical chemistry
There were no notable intergroup differences in either sex.

H. NECROPSY
Organ weights
There were no notable intergroup differences in either sex.

Gross pathology
Unilateral dilatation of the kidneys was seen in 2/5 high dose males compared to 0/5 Controls. This is a common finding in rats of this age and not considered related to treatment with glyphosate. There were no notable intergroup differences for females.

Histopathology
Unilateral papillary necrosis was seen in the kidney in 1/5 high dose males and urothelial hyperplasia was seen in the kidney in 2/5 High dose males, both compared to 0/5 in Controls. These lesions are relatively common in rats of this age and strain and are not considered to be related to treatment with glyphosate. Pelvic dilation was seen in 3/5 High dose males compared to 0/5 in Controls. There were no other notable intergroup differences for males or females.

Table 6.3.3-8: Glyphosate – 3 Week Toxicity Study in Rats with Dermal Administration [redacted] 1993): Summary of selected haematology data

Parameter		Group/Dose level [mg/kg bw/day]			
		1/0 ♂	**2/1000 ♂**	**1/0 ♀**	**2/1000 ♀**
Kidneys	No abnormality detected	3	1	4	5
	Localised urothelial hyperplasia (Grade +/-)	0	2	0	0
	Total incidence for score expanded finding	0	2	0	0
	Basophilic tubules (Grade +/-)	0	1	0	0
	Total incidence for score expanded finding	0	1	0	0
	Pelvic dilatation (Grade +/-) (Grade ++) (Grade ++1-)	0 0 0	1 1 1	1 0 0	0 0 0
	Total incidence for score expanded finding	0	3	1	0
	Localised papillary necrosis	0	1	0	0
	Nephropathy (Grade +/-) (Grade +)	1 1	1 0	0 0	0 0
	Total incidence for score expanded finding	2	1	0	0

III. CONCLUSIONS

No effect on mortality or clinical signs could be observed. Findings in body weight changes or food/water consumption were not consistent and considered to be not treatment-related. The skin assessment showed a minor reaction to the treatment of glyphosate. Haematology, clinical chemistry or organ weights revealed no notable intergroup differences in either sex. Histopathological examination did not reveal any finding that could be attributed to dosing with glyphosate.

In conclusion, following dermal administration of glyphosate to Sprague-Dawley rats for 3 weeks at a dose level of 1000 mg/kg bw/day, there was no evidence of systemic toxicity. A mild transitory irritant effect was noted at the dosing site.

Assessment and conclusion by applicant:
In this study, a group of 5 male and 5 female Sprague-Dawley rats were dosed daily with glyphosate via the dermal route of application, for a period of ca 6 h per day for 3 weeks. The study was in general compliance with OECD 410 (1981) and GLP.

In conclusion, following dermal administration of glyphosate to Sprague-Dawley rats for 3 weeks at a limit dose level of 1000 mg/kg bw/day, there was no evidence of systemic toxicity. A mild irritant effect was noted at the dosing site.

Therefore, the NOAEL for dermal administration in rats under the conditions of this study can be set at 1000 mg/kg bw/day.

Assessment and conclusion by RMS:
The study is considered acceptable and the assessment by the applicant is agreed including the proposed NOAEL for systemic toxicity of 1000 mg/kg bw/day, the only dose tested. This conclusion is in agreement with the previous assessments (DAR and RAR). Mild irritant effects (erythema and desquamation) were noted at the dosing site in the animals of the glyphosate-treated group. No NOAEL for local effects could be derived. The only dose tested (1000 mg/kg bw/day) is considered the LOAEL for local effects as mild skin irritation (erythema and desquamation) was noted at this dose level.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"*Re-evaluation by the RMS revealed that the study on rats by [redacted] (1993, TOX9552367) […]may be still considered acceptable"*
In the original assessment in the DAR, the following was concluded:
" No evidence of any systemic effects of treatment was elicited neither by in life observations, laboratory investigations nor post mortem examinations. Thus, the NOEL for systemic toxicity was 1000 mg/kg bw/day in this limit test. In contrast, mild transitory irritant effects (erythema and desquamation) were noted at the dosing site in the animals of the glyphosate-treated group.

B.6.3.3.3. Dermal repeated dose toxicity – study 3

Data point	CA 5.3.3/004
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical ([redacted] Repeated dose twenty-eight- Day dermal toxicity study in rabbits (Part 1, Study Report)
Report No	[redacted]214/94
Document No	Not reported
Guidelines followed in study	No guideline followed; in general compliance with OECD guideline 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	None
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Data point	CA 5.3.3/005
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical ([redacted] Repeated dose twenty-eight-Day dermal toxicity study in rabbits (Part 2, Individual data)
Report No	[redacted] 214/94
Document No	Not reported
Data point	CA 5.3.3/006
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical ([redacted] Repeated dose twenty-eight-Day dermal toxicity study in rabbits (Part 3, Individual data)
Report No	[redacted] 214/94
Document No	Not reported
Acceptability/Reliability	**Conclusion GRG**: Valid, Category 2a **Conclusion AGG**: Acceptable

Executive Summary
The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied for a 6-hour period on five consecutive days per week over 4 weeks. For application the solid test substance was mixed with water resulting in a 50 % (w/v) solution, and spread evenly over the application site.

There were no mortalities and no treatment-related signs of systemic toxicity. Very slight erythema was noted in one high-dose male and one low-dose female. However, this effect is not considered biologically significant and was not seen in the histopathological examination.
There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one high-dose male and one low-dose female. There were no treatment-related systemic signs of toxicity.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate technical
Description: White powder
Lot/Batch#: 39730494
Purity: 99.6 %
Stability of test compound: Not reported

2. Vehicle and/ or positive control: Water

3. Test animals:

Species: Rabbit

Strain:	New Zealand
Source:	[redacted]
Age:	Young, adult
Sex:	Male and female
Weight at dosing:	♂ 2285 – 3330 g; ♀ 2195 – 3115 g
Acclimation period:	7 days
Diet/Food:	Altromin Rabbit Chow, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 18 ± 2 °C Humidity: Not reported Air changes: 10 / hour 12 hours light / dark cycle

B: Study design and methods

In life dates: 1994-05-23 to 1994-06-20

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Five rabbits per sex per dose received daily dermal applications of 0, 500, 1000 or 2000 mg/kg bw/day, five days per week for a total of 20 applications.

Two days prior to the first application about 15 % of the skin of the dorsal back of the animals was clipped free of hair. The clipping was repeated weekly thereafter.

For each application the test substance was mixed with water to give a final concentration of 50 % (w/v) of glyphosate. Each dose was spread evenly over about 10 % of the body surface area and covered with a occlusive dressing made of polyethylene material that was secured by hypoallergenic Leucoplast tape. After an exposure period of six hours the dressings were removed and the application site was cleaned with hand soap, water and clean, absorbent paper pads. Applications were performed once daily, five days per week for a total of 28 days.

Clinical observations
A check for mortality, clinical signs of toxicity, and general appearance and behaviour, as well as a quantitative assessment of food and water intake was made twice daily. The applications sites were assessed for signs of irritation once daily.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed at weekly intervals during the pre-test and study periods and before sacrifice.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on all rabbits at termination.

The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count, differential leukocyte count, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), coefficient of variation of erythrocyte volume (RDW), platelet volume distribution (PDW), mean platelet volume (MPV), thrombocrit (volume % of platelets), aspartate amino transferase (AST), alanine aminotransferase (ALT), blood urea nitrogen, total protein, glucose, albumin, total bilirubin, creatinine, inorganic phosphorus, calcium, sodium, potassium and chloride.

Sacrifice and pathology

All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, brain, heart, kidneys, liver, lung, spleen, stomach, thymus, and testes. The organ-to-brain weight ratios were calculated.

Tissue samples were taken from the following organs and preserved in buffered formalin: Treated and untreated skin, adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, stomach, testes, and thyroid. Histopathological examinations were performed on all collected tissues from the control and high-dose animals, as well as from abnormal tissues of animals from the low- and mid-dose groups.

Statistics
Body weights, haematological and clinical chemistry parameters, absolute and relative organ weights and histopathology data of treated animals were compared with control animals. Body weight, food consumption and haematology and clinical chemistry parameters were analysed by t-test. Histopathology data were analysed by Fisher´s exact test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL AND DERMAL OBSERVATIONS
There were no signs of systemic toxicity noted in any animal of any dose group.

Signs of dermal irritation consistent of very slight erythema without oedema were observed in one high-dose male and one low dose female. The erythema lasted from day 7 to 20 for the male, and for 5 days for the female. Other dermal findings were irritation from the tape, scabs cracked and/or flaking skin. These findings occurred sporadically throughout the groups, irrespective of dose.

C. BODY WEIGHT
There were no statistically significant differences observed in body weights between the control and treated groups. Body weight gain was significantly decreased in males of the mid dose group (244.4 gram at the mid dose vs 369.8 gram in the control group; $P<0.05$), however as a dose-response relationship is lacking, this change is not considered treatment-related.

D. FOOD CONSUMPTION
There were statistically significant differences in food consumption between the control and the group treated at 1000 mg/kg bw/day during week 3 and 4. However, the observed differences were considered unrelated to treatment as no dose-response was observed. See Table below:

Table 6.3.3-1: Glyphosate technical ([redacted] Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits [redacted] 1994): Group mean food consumption values [g] and standard deviations (SD)

Dose level [mg/kg bw/day]	Week 1	Week 2	Week 3	Week 4
Males				
0	311.4 ± 38.0	306.8 ± 20.9	286.8 ± 5.8	303.8 ± 28.5
500	319.8 ± 23.7	278.2 ± 30.5	280.8 ± 35.2	267.6 ± 33.3
1000	292.0 ± 44.2	261.6 ± 48.3	**244.6* ± 25.3**	**240.0** ± 25.8**
2000	279.4 ± 42.2	269.0 ± 40.3	260.2 ± 35.4	276.8 ± 30.5
Females				

Table 6.3.3-1: Glyphosate technical [redacted] [redacted] Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits [redacted] 1994): Group mean food consumption values [g] and standard deviations (SD)

Dose level [mg/kg bw/day]	Week 1	Week 2	Week 3	Week 4
0	301.8 ± 51.8	289.8 ± 44.8	271.8 ± 33.5	272.2 ± 39.9
500	317.4 ± 26.1	303.8 ± 19.6	289.6 ± 19.2	291.6 ± 25.5
1000	323.6 ± 16.0	317.4 ± 33.2	311.4 ± 23.4	292.2 ± 17.1
2000	315.2 ± 41.7	297.4 ± 43.1	295.0 ± 32.3	289.6 ± 43.6

* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$)

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology
No treatment-related effects were detected in the haematological parameters measured.

In females of the mid-and high-dose group Mean Corpuscular Volume of Platelets (MPV) and Coefficient of Variation of Platelets Volume (PDW) values were significantly lower when compared to controls. However, the values were within the historical control range for female NZW rabbits of this age (not shown in this study report). Thus, these changes were not considered treatment-related (see below). The RMS considers the changes in MPV and PDW as non-adverse as changes are slight.

Blood chemistry
There were no treatment-related effects. The incidental significant changes observed with regard to urea level in high dose males were within the historical control range (not shown in this study report) of the testing facility (see table below). The RMS considers the increased urea levels at the top dose as not treatment-related as no dose-response is observed and changes are slight.

Table 6.3.3-2: Glyphosate technical ([redacted] Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits ([redacted] 1994): Group mean haematological and blood chemical values and standard deviations (SD)

Dose level [mg/kg bw/day]	MPV [fL]	PDW [fL]	Urea [mmol/L]
Males			
0	7.48 ± 0.28	5.80 ± 1.10	7.80 ± 0.66
500	7.94 ± 0.62	6.80 ± 1.57	8.72 ± 1.68
1000	7.64 ± 0.42	6.20 ± 1.04	7.64 ± 0.50
2000	7.48 ± 0.04	5.60 ± 0.42	**9.82** ± 1.02**
Females			
0	8.96 ± 0.85	9.70 ± 1.86	10.24 ± 1.09
500	8.15 ± 0.99	7.50 ± 2.71	9.70 ± 0.53
1000	**7.62* ± 0.13**	**6.20* ± 0.57**	10.58 ± 0.39

Table 6.3.3-2: Glyphosate technical ([redacted] Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits [redacted] 1994): Group mean haematological and blood chemical values and standard deviations (SD)

Dose level [mg/kg bw/day]	MPV [fL]	PDW [fL]	Urea [mmol/L]
2000	**7.46* ± 0.27**	**5.10*** ± 0.65**	9.96 ± 1.31

MPV: Mean platelet volume; PDW: Platelet volume distribution (coefficient of variance of platelets volume);
* Significantly different from control group ($p < 0.05$);
** Significantly different from control group ($p < 0.01$);
*** Significantly different from control group ($p < 0.001$)

I. NECROPSY

Organ weights

Absolute testes weights were statistically increased in high dose males but as relative testes to brain weights did not differ between groups this was not considered treatment-related.

Table 6.3.3-3: Glyphosate technical ([redacted] Repeated Dose Twenty-eight-Day Dermal Toxicity Study in Rabbits [redacted] 1994): Group mean absolute and relative to brain testes weight values and standard deviations (SD)

Dose level [mg/kg bw/day]	Absolute weight [g]	Relative weight [g/1g brain wt.]
0	3.88 ± 0.327	0.4310 ± 0.0215
500	3.70 ± 1.255	0.3744 ± 0.1211
1000	4.36 ± 0.658	0.4692 ± 0.0730
2000	**4.52* ± 0.497**	0.4618 ± 0.0773

* Significantly different from control group ($p < 0.05$)

Gross pathology

There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology

There was an increased incidence in high dose males versus control animals of erosions in the stomach (4/5 high dose versus 1/5 control; non-significant) and interstitial nephritis (3/5 high dose versus 1/5 control; non-significant) but these findings were not considered treatment-related lesions. The RMS agrees that both findings are not considered treatment-related when also taking into account the severity of the pathological findings. The erosions in the stomach were scored as moderate in controls (one animal) and one as slight and three as moderate in the top dose animals. As stomach erosion are regularly seen in laboratory animals and considering the dermal route of administration, this is not considered treatment-related. For the kidney, the one incidence of interstitial nephritis in controls was scored as moderate, whereas the three incidences at the top dose were conserved as slight. In one high dose female, a slight acanthosis, hyperkeratosis and perivascular infiltration was observed, which was not seen in the females of the controls group or in any of the males (high dose or control).

III. CONCLUSIONS

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one high-dose male and one low-dose female. There were no compound-related gross and microscopic findings noted.

Assessment and conclusion by applicant:

In this study, the toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied for a period of 6 hours a day, five days per week for 4 weeks. The study was conducted according to OECD 410 (1981) and in compliance with GLP.
Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one of five high-dose males and one low-dose female. There were no treatment-related systemic signs of toxicity. Thus, the NOAEL is considered to be 2000 mg/kg bw/day.

Assessment and conclusion by RMS:

The study is considered acceptable and the assessment by the applicant is agreed including the proposed NOAEL for systemic toxicity of 2000 mg/kg bw/day, the highest dose tested. This conclusion is in agreement with the previous assessments (DAR and RAR). Slight dermal irritation were reported in one of the five top-dose males and one low-dose female. Local effects were limited to a very slight erythema noted in one high-dose male and one low-dose female. Only the slight dermal irritation in the top dose male is considered for setting a NOAEL for local skin irritation. The local effects in the low-dose female is not further considered as no dose-response was observed. Therefore, the RMS proposed a NOAEL for local effects of 1000 mg/kg bw/day based on the skin irritation observed in high dose males.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"*Re-evaluation by the RMS revealed that the study […] by […..]* [redacted] *(1994, TOX9650151) on rabbits may be still considered acceptable"*
In the original assessment in the DAR, it was concluded that there were no treatment-related adverse effects observed in the study and that the highest dose level of 2000 mg/kg bw/day can be considered the NOAEL.

B.6.3.3.4. Dermal repeated dose toxicity – study 4

Data point:	CA 5.3.3/007
Report author	[redacted]
Report year	1985
Report title	Subacute dermal toxicity (for 21 days in rabbits) of glyphosate (technical)
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not reported, comparable to OECD 410 (1981); however a 14-day reversal interval was applied before sacrifice.
GLP	No
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	In a 21-day dermal toxicity study, glyphosate (purity not reported) was dermally applied to the intact skin of New Zealand White rabbits for 6 hours per day. The dose levels were 0, 500, 1000, and 2000 mg/kg bw/day and the groups consisted of 3 male and 3 female rabbits per group. Treatment was performed 5 days per week for 3 consecutive weeks. Treatment was followed by a 14-day recovery period prior to sacrifice. Animals were observed daily for clinical signs of toxicity and irritation. Food consumption was calculated daily and body weights were determined weekly. Laboratory investigations (haematology, clinical chemistry, and urinalysis) were performed on days 0, 21, and 35. At study termination, all animals were subjected to a gross and histopathological examination.
Short description of results:	There were no mortalities and no treatment-related signs of clinical toxicity. There were no signs of dermal irritation. Body weight and food consumption were not affected by treatment. Laboratory

	investigations and gross and histopathological examinations did not reveal any treatment-related effects. The No Observed Adverse Effect Level (NOAEL) was established at 2000 mg/kg bw/day based on the lack of effects.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was considered supportive due to serious reporting deficiencies in the Monograph (2000) and not accepted in the RAR (2015). Test substance purity and batch number or study details like study number were not reported. Additionally, a statistical analysis of the results was not reported. Therefore and since the study report is not available to GRG, this study is not considered to be reliable by GRG. **Conclusion GRG:** The study is considered unacceptable, category 4b. **Conclusion AGG**: The study report has been made available to AGG by BVL. The RMS has evaluated the study and agrees with the previous conclusion that the study is not considered acceptable due to missing information on the batch and purity of the test substance. The study design is comparable to OECD 410 (1985), however, sacrifice was after a 14-day recovery period for all animals, which is not in agreement with the OECD guideline. The RMS agrees with the results reported above and with the conclusion that there were no treatment-related effects. However, no NOAEL is proposed as the study is not considered acceptable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. The former RMS (BVL) has made the study report available to the current RMS.

B.6.3.3.5. Dermal repeated dose toxicity – study 5

Data point	CA 5.3.3/008
Report author	[redacted]
Report year	1982
Report title	21-Day Dermal Toxicity Study in Rabbits
Report No	[redacted]81-195
Document No	Not reported
Guidelines followed in study	No guideline followed; in general compliance with OECD 410 (1981)
Deviations from current test guideline (OECD 410, 1981)	- The purity of the test substance is not reported. - The application area in the high-dose group was about 1.5–2 times higher than the recommended 10% of the body surface area. - With the highest test dose of 5000 mg/kg bw/day, the limit dose of 1000 mg/kg bw/day is far exceeded.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG**: Valid, Category 2a **Conclusion AGG**: The study is considered unacceptable as the purity and stability of the test substance is not reported.

Executive Summary

The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of 5 male and 5 female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. The abrading was accomplished by producing shallow incisions (not deep enough to cause bleeding) with the blunt end of a scalpel. For application the solid test substance was moistened with an appropriate amount of water, and spread evenly over the application site. It has to be noted that the surface areas covered (i.e. 1 – 2%, 5 – 10% and 15 – 20% body surface area for the low, mid- and high-dose group, respectively) were below and above the area of 10% recommended by actual guidelines. Due to the higher exposed surface area in the high dose group, it has to be considered that more test substance can be absorbed through the skin and could be therefore systemically available.

There were no mortalities and no treatment-related signs of systemic toxicity. There were also no signs of dermal irritation observed in the control, low- and mid-dose group. At 5000 mg/kg bw/day slight dermal irritation consisting of barely perceptible to slight erythema and oedema was noted. However, this effect is considered not to be of biological significance and no signs of irritation were seen in the histopathological examination.

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment groups. There were no treatment-related systemic signs of toxicity.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch#:	NBP 1992026
Purity:	Not reported
Stability of test compound:	Not reported

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	[redacted]
Age:	Young, adult
Sex:	Male and female
Weight at dosing:	♂ 2359 – 2883 g; ♀ 2344 – 2955 g
Acclimation period:	14 – 16 days
Diet/Food:	Purina Certified Rabbit Chow #5322 (Ralston Purina Company, Missouri, USA), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh 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: 1981-07-28 to 1981-08-19

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Ten rabbits per sex per dose received daily dermal applications of 0, 100, 1000 or 5000 mg/kg bw. The dose groups were further divided in halves. One half received the treatment on intact skin, the other half on abraded skin. Abrasion was done twice per week immediately prior to test substance application by producing shallow incisions (not deep enough to cause bleeding) with the blunt end of a scalpel blade.

The day prior to the first application about 30 % of the skin of the back of the animals was clipped free of hair. During the study rabbits were shaved as needed.

For each application the test substance was moistened with an appropriate amount of physiological saline. Each dose was spread evenly over the maximum body surface area possible (see table below) covered with a semi-occlusive dressing. The entire area was wrapped with Saran wrap and Elastoplast tape. To avoid oral ingestion of the test article, collars were applied during the conditioning period (Day -7) and remained on each rabbit for the duration of the study. After an exposure period of six hours the dressings were removed and the application site was cleaned with tepid tap water and dried with paper towels. Applications were performed once daily, five days per week for three consecutive weeks. Individual doses were adjusted weekly based on the body weight determined at the beginning of each study week.

Table 6.3.3-11: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Application details for repeated dermal applications

Dose group [mg/kg bw/day]	Number of animals				Volume of physiological saline used for moistening [mL]	Total percent of the body surface covered by test substance [%]
	Intact skin		Abraded skin			
	♂	♀	♂	♀		
0	5	5	5	5	-	-
100	5	5	5	5	0.2	1 – 2
1000	5	5	5	5	1.5 – 2.0	5 – 10
5000	5	5	5	5	8.0 – 9.0	15 – 20

It has to be noted that the application area according to current guidelines (OECD and EC) should be about 10% of the body surface. Thus, the body surface covered with test material in the 100 mg/kg bw/day dose group is lower than recommended, whereas the treatment-area in the high dose group is about 1.5 – 2 times higher than recommended. Due to the higher exposed surface area in the high-dose group a higher amount of test substance can be absorbed and therefore potentially be systemically available.

Clinical observations
A check for mortality was made twice daily. Observations for clinical signs of toxicity and behavioural changes were made once daily on all animals. The applications sites were assessed for signs of irritation.

Body weight
Individual body weights were recorded at twice weekly during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed daily for each individual animal by visual inspection.

Haematology and clinical chemistry

Haematological and blood chemical investigations were performed on 5 rabbits per sex and dose group with intact and abraded skin on day 21 after an overnight fast. Blood was obtained via puncture of the main ear artery.

The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, reticulocyte count, platelet count, total leukocyte count, differential leukocyte count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), creatine kinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin (calculated), total bilirubin , creatinine, lactate dehydrogenase, total cholesterol, inorganic phosphorus, calcium, sodium, potassium and chloride.

Sacrifice and pathology
All animals sacrificed at scheduled termination were subjected to a gross pathological examination. Any macroscopic findings were recorded.

The following organ weights were determined: Adrenals, gonads, heart, kidneys, liver, pituitary , testes and thyroid (with parathyroid).

Tissue samples were taken from the following organs and preserved in buffered formalin: Treated and untreated skin (3 samples each), adrenals, bone & bone marrow (sternum), brain (at three levels), colon, duodenum, eyes with Harderian gland, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs with main stem bronchi, mammary gland, lymph nodes (mediastinal, mesenteric and regional when applicable), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, salivary gland (mandibular with submandibular lymph node), sciatic nerve, seminal vesicles, spinal cord (cervical), spinal cord and vertebrae (lumbar), spleen, stomach, testes with epididymis, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix) and vagina.

Histopathological examinations were performed on the following tissues: Treated and untreated skin, liver, kidney, gonads (ovary, testis with epididymis, uterus/prostate and seminal vesicle) and any gross lesions.

Statistics
Terminal body weights, haematological and clinical chemistry parameters, absolute and relative organ weights were analysed by one-way analysis of variance, Bartlett´s test for homogeneity of variance and appropriate t-test (for equal and unequal variances).

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
A number of incidental findings were observed in some animals in all dose groups. The most frequent signs were soft stool, diarrhoea, mucoid diarrhoea and ocular and nasal discharge.

No signs of dermal irritation were observed in the control, low- and mid-dose group. In the high-dose group at 5000 mg/kg bw/day doubtful or barely perceptible to very slight erythema and doubtful or barely perceptible oedema were noted. Dermal effects were observed as early day 2 with increasing number of animals showing the effects after additional exposures. There were no differences between the animals with intact and abraded skin.

C. BODY WEIGHT
There were no statistical significant differences observed in body weights or body weight gains between the control and treated groups (with abraded and intact skin).

D. FOOD CONSUMPTION
There were no major differences in food consumption between the control and the treated groups.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
No treatment-related effects were detected in the haematological parameters measured.

There were some statistical significance differences in some parameters. However, these were incidental as a dose-response relationship is lacking and therefore considered to be biologically insignificantly (see table below).

Blood chemistry

In the study report it was concluded that there were no treatment-related effects. The incidental significant changes observed were considered not to be biologically significant (see Table 6.3.3-). The RMS agrees that the changes in glucose levels in females treated at 100 and 5000 mg/kg bw/day and LDH levels in top dose males (not significant) and top dose females (significant) are not toxicologically relevant as these were due to large biological variation. However, the significantly increased sodium levels in males treated at 5000 mg/kg bw/day are considered treatment-related by the RMS, however, the clinical relevance remains unclear.

Table 6.3.3-2: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Group mean haematological and blood chemical values and (± SD)

Dose level [mg/kg bw/day]	Hb [g/dL]	Haematocrit [%]	Sodium [mEq/L]	Glucose (mg/dL)	LDH (IU/L)
Males					
0	11.3 ± 0.68	34.1 ± 1.78	143 ± 1.3	121 ± 21.6	258 ± 154.5
100	**12.5* ± 0.63**	**37.3* ± 1.92**	144 ± 2.1	134 ± 14.1	169 ± 115.9
1000	11.4 ± 0.46	34.2 ± 1.41	145 ± 5.1	149 ± 34.7	291 ± 198.7
5000	11.7 ± 0.61	35.5 ± 1.13	**146* ± 2.5**	125 ± 6.1	70 ± 72
Females					
0	11.5 ± 0.43	35.4 ± 2.14	142 ± 2.7	102 ± 16.2	189 ± 125.9
100	11.4 ± 0.50	34.3 ± 0.85	142 ± 1.5	**137** ± 18.7**	149 ± 109.1
1000	11.5 ± 0.50	34.5 ± 1.28	143 ± 1.9	123 ± 17.1	258 ± 204.4
5000	11.7 ± 0.42	34.4 ± 1.55	144 ± 1.6	**129* ± 4.7**	**28* ± 6.2**

LDH: Lactate dehydrogenase;

* Significantly different from control group ($p < 0.05$);

** Significantly different from control group ($p < 0.01$)

I. NECROPSY

Organ weights

Except for the statistically increased relative kidney weight observed in females at 5000 mg/kg bw/day (see table below), there were no treatment-related effects on absolute and relative organ weights noted. Since no histopathological changes were observed in the kidneys of high-dose females, the increase in relative kidney weights is considered to be of no toxicological relevance by the study authors. However, the RMS considers the increased kidney weights in top-dose females as treatment-related and adverse as changes in both absolute and relative kidney weight are >10% compared with controls.

Table 6.3.3-3: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Group mean absolute and relative kidney weights and standard variations (± SD)

Dose level [mg/kg bw/day]	Absolute organ weight [g]		Relative organ weight [%]	
	Kidney		Kidney	
	♂	♀	♂	♀
0	17.00 ± 2.281	16.41 ± 2.037	0.58 ± 0.078	0.55 ± 0.040

Table 6.3.3-3: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Group mean absolute and relative kidney weights and standard variations (± SD)

Dose level [mg/kg bw/day]	Absolute organ weight [g]		Relative organ weight [%]	
	Kidney		Kidney	
	♂	♀	♂	♀
100	16.38 ± 1.371	15.26 ± 2.260	0.57 ± 0.067	0.54 ± 0.069
1000	18.15 ± 2.653	16.16 ± 2.449	0.59 ± 0.067	0.54 ± 0.048
5000	16.77 ± 2.016	18.14 ± 1.757 (+10.5%)	0.60 ± 0.097	**0.63* ± 0.072** (+14.5%)

* Significantly different from control group ($p < 0.05$)

Gross pathology
There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology
There were no treatment-related lesions observed in any dose group.

Microscopic evaluation of treated skin samples demonstrated only mild inflammatory cell infiltration and trace necrosis in the 1000 mg/kg bw/day group. However, in untreated skin samples of three rabbits from the 1000 mg/kg bw/day group from one rabbit of the high-dose group there was also mild necrosis, indicating that this lesion was incidental and unrelated to treatment. The lesions in treated and untreated skins of the control and test substance groups were similar indicating that the effects were not related to glyphosate treatment.

Trace/mild seminiferous tubule degeneration observed in the testis was not dose related in either incidence or severity and was considered unrelated to treatment. Other lesions observed in kidney, liver, lung, ovary, lymph node, salivary gland and skin (non-application site) were considered incidental or spontaneous (see tables below). In general there were no major differences between the treatment groups of intact and abraded skin.

Table 6.3.3-4: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Histopathological findings in rabbits treated dermally on intact skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Cytoplasmic vacuolation (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Interstitial lymphocytic infiltrates (mild)	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Interstitial inflammation (mild)	0/5	1/5	1/5	0/5	0/5	0/5	2/5	0/5
Infarct (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Mineralisation (trace)	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	1/5	0/5	1/5	1/5
Liver								
Granuloma (moderate)	0/5	0/5	1/5	1/5	1/5	0/5	0/5	0/5
Mononuclear cell infiltration (trace)	1/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5
Mononuclear cell infiltration (mild)	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
Mononuclear cell infiltration (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Necrosis (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Lung								
Abscess (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5

Table 6.3.3-4: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Histopathological findings in rabbits treated dermally on intact skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Lymphocytic infiltration (mild)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Pneumonia (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Pneumonia (moderate)	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Congestion (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Congestion (moderate)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Oedema (moderate)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis								
Seminiferous tubule degeneration (trace)	1/5	--	3/5	--	2/5	--	3/5	--
Seminiferous tubule degeneration (mild)	2/5	--	2/5	--	0/5	--	1/5	--
Dilated tubules (moderate)	0/5	--	1/5	--	0/5	--	0/5	--
Ovaries								
Mineralisation (trace)	--	1/5	--	0/5	--	0/5	--	0/5
Salivary gland								
Abscess (moderate)	--	1/5	--	0/5	--	0/5	--	0/5
Skin (non-application site)								
Dermatitis (moderate)	0/5	--	0/5	--	1/5	--	0/5	--
Skin, treated								
Inflammation (trace)	1/5	3/5	2/5	3/5	2/5	5/5	3/5	2/5
Inflammation (mild)	2/5	1/5	2/5	1/5	1/5	0/5	0/5	3/5
Necrosis (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Skin, untreated								
Inflammation (trace)	4/5	1/5	4/5	2/5	3/5	2/5	3/5	2/5
Inflammation (mild)	1/5	3/5	0/5	3/5	1/5	2/5	0/5	3/5
Necrosis (mild)	0/5	0/5	0/5	0/5	3/5	0/5	1/5	0/5

* Number of animals affected / total number of animals;
- Not applicable

Table 6.3.3-5: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Histopathological findings in rabbits treated dermally on abraded skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Interstitial inflammation (trace)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (mild)	1/5	0/5	1/5	1/5	1/5	0/5	0/5	1/5
Interstitial inflammation (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5
Infarct (moderate)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	2/5	1/5	0/5	0/5
Liver								
Mononuclear cell infiltration (trace)	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Mononuclear cell infiltration (mild)	1/5	0/5	1/5	2/5	4/5	1/5	1/5	1/5
Necrosis (mild)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Lung								
Pneumonia (moderate)	0/5	--	1/5	--	1/5	--	0/5	--

Table 6.3.3-5: 21-Day Dermal Toxicity Study in Rabbits [redacted] 1982): Histopathological findings in rabbits treated dermally on abraded skin*

Dose level [mg/kg bw/day]	0		100		1000		5000	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Lymph node, prefemoral								
Lymphadenitis (mild)	--	0/5	--	0/5	--	0/5	--	1/5
Testis								
Seminiferous tubule degeneration (trace)	2/5	--	1/5	--	2/5	--	2/5	--
Seminiferous tubule degeneration (mild)	2/5	--	3/5	--	2/5	--	2/5	--
Ovaries								
Mineralisation (trace)	--	0/5	--	0/5	--	1/5	--	0/5
Mineralisation (mild)	--	0/5	--	0/5	--	1/5	--	0/5
Atretic follicles (mild)	--	0/5	--	1/5	--	0/5	--	0/5
Skin, treated								
Inflammation (trace)	2/5	2/5	3/5	2/5	4/5	2/5	2/5	1/5
Inflammation (mild)	0/5	3/5	2/5	1/5	0/5	3/5	3/5	4/5
Skin, untreated								
Inflammation (trace)	3/5	3/5	2/5	4/5	2/5	2/5	1/5	3/5
Inflammation (mild)	1/5	1/5	3/5	1/5	3/5	2/5	4/5	2/5

* Number of animals affected / total number of animals

III. CONCLUSIONS

There were no mortalities and no treatment-related signs of systemic toxicity. There were also no signs of dermal irritation observed in the control, low- and mid-dose group. At 5000 mg/kg bw/day slight dermal irritation consisting of barely perceptible to slight erythema and oedema was noted. However, this effect is considered not to be of biological significance and no signs of irritation were seen in the histopathological examination.

There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment groups. There were no treatment-related systemic signs of toxicity. No test article-related macroscopic or microscopic lesions were observed.

Assessment and conclusion by applicant:
In this study, the toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. The study was conducted following a testing regime in general compliance with OECD 410 (1981) and with GLP.

Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment groups. There were no treatment-related systemic signs of toxicity of biological significance. Thus, the NOAEL is considered to be 5000 mg/kg bw/day.

In light of an *in vitro* dermal absorption of glyphosate with rabbit skin following OECD 428 [redacted] 2012; CA 5.8.2/014), demonstrating 2.66 % bioavailability via the skin exposure, the systemic NOAEL in this study is 133 mg/kg bw/day, de

Assessment and conclusion by RMS:
Although the study has been accepted in the previous assessments, the current RMS does not considered this study acceptable as the purity and stability of the test substance are not provided in the study report.
Therefore no NOAEL is proposed for this study. Regarding the assessment of the study, the RMS considers the increased kidney weights (absolute and relative) in females at the highest dose level of 5000 mg/kg bw/day as treatment-related and adverse. In addition, increased sodium levels were noted in males treated at the top dose. Regarding local skin findings, only a slight dermal irritation was noted at 5000 mg/kg bw/day.

In the previous assessment in the RAR (2015), the following was stated by the RMS DE:
"*Re-evaluation by the RMS revealed that the study […] by* [redacted] *(1982, TOX9552366) […..] on rabbits may be still considered acceptable"*
In the original assessment in the DAR, it was concluded that there was no systemic toxicity observed up to the highest dose. Therefore, the highest dose level of 5000 mg/kg bw/day can be considered the NOAEL. In contrast, rather slight dermal irritation occurred at this top dose level.

B.6.3.3.6. Inhalation – 14-day repeated dose toxicity – study 1

Data point:	CA 5.3.3/009
Report author	[redacted]
Report year	1985
Report title	Report on subacute inhalation toxicity in rats (14 days) of glyphosate (technical)
Report No	Not reported
Document No	Not reported
Guidelines followed in study	Not applicable
GLP	No, pre-GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	Four groups of 5 male and 5 females Wister rats (bred at Indian Institute of Toxicology, Bombay, India) were exposed to an atmosphere containing glyphosate (purity not stated) in propylene glycol for 6 hours per day, 5 days per week for two weeks. There were one low and one high dose group and two intermediate dose groups. One of the latter groups was sacrificed 14 days after the treatment period had been finished (reversal group). Two control groups of the same size were also included, one of them being exposed to filtered air only and the other to an atmosphere containing the vehicle propylene glycol. Glyphosate was mixed with the vehicle and nebulised by using compressed air. The animals were exposed in a dynamic inhalation chamber by mouth and nose route by restraining them in polypropylene tubes. The target, the nominal and the actually measured mean concentrations for the various groups are given in the table below.

Table 6.3.3-1: Report on subacute inhalation toxicity in rats (14 days) of glyphosate (technical) [redacted] 1985): Mean concentrations [mg/L air] of the test material or the vehicle

Dose group	Target concentration	Nominal concentration	Measured concentration
Control (filtered air)	0	0	0
Vehicle control	5.0	16.5	5.6
Low dose	0.25	0.90	0.28
Intermediate dose	1.0	3.01	0.93

	<table><tr><td>Intermediate dose (reversal)</td><td>1.0</td><td>3.10</td><td>0.90</td></tr><tr><td>High dose</td><td>4.0</td><td>11.7</td><td>3.8</td></tr></table> With the exception of the exposure periods, animals were housed five per cage and had free access to food and drinking water. The rats were examined individually before, during and after exposure for any signs of toxicity. Food consumption was recorded daily and body weight was determined at pretest and then on every 4th day during the study and on the day of termination. Blood samples for haematological (red and white blood cell parameters, thrombocyte count and prothrombin time) and clinical chemistry (total serum protein, blood urea nitrogen, glucose, alanine aminotransferase (ALAT) and alkaline phosphatase (AP)) investigations were taken before first exposure and after one week from some animals of each group and from all animals (except the recovery group) after the last exposure. From reversal group rats, blood was obtained at termination. Urinalysis was also performed. All rats were necropsied and the following organs were removed and weighed: adrenals, gonads, heart, kidneys, liver, and spleen. These organs as well as aorta, brain, eyes, intestines, larynx, lungs, lymph nodes (axillary and mesenteric), nose, oesophagus, pancreas, pituitary, seminal vesicle, stomach, thyroid, trachea, urinary bladder and uterus were examined microscopically.
Short description of results:	Mass median aerodynamic diameters of all atmospheres were within the respirable range of 0 - 7 µm on all occasions. The temperature, humidity, oxygen concentration and air flow were similar for all groups. There was no mortality during the treatment or the recovery periods. Neither treatment-related clinical signs nor effects on body weight or food consumption were observed. In the vehicle control group and in all groups exposed to glyphosate, salivation, lacrimation, redness to nose and urination were observed during exposure. No change in the rate of respiration was seen. No adverse effects of treatment on haematology, clinical chemistry and urinalysis were noted in any of the groups. No significant changes in organ weights or upon gross examination were seen. Histopathology did not reveal evidence of adverse effects. Up to the highest concentration tested of approx. 3.8 mg/L air (mean measured concentration) repeated inhalative exposure of Wistar rats to an aerosol containing glyphosate did not lead to any local (respiratory) or systemic toxicity. Therefore, the NOAEC for rats under the conditions of this study was 3.8 mg/L.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Monograph (2000): The study was considered supportive since an effect dose was lacking. In addition, there were some reporting deficiencies. Furthermore, statistical analysis of the results obtained was either not performed or not reported. RAR (2015): The study was considered unacceptable due to serious reporting deficiencies, e.g., absence of information on batch and purity of the test material. Therefore and since the study report is not available to GRG, this study is considered invalid by GRG. **Conclusion GRG:** The study is considered unacceptable, category 4b. **Conclusion AGG:** The study report has been made available to AGG by BVL. The RMS has evaluated the study and agrees with the previous conclusion that the study is not considered acceptable due to serious reporting deficiencies, e.g.

	absence of statistical analysis, and purity and batch number of the test substance not reported. The RMS agrees with the results of the study as reported above. There were no signs of local or systemic toxicity up to the highest dose. However, no NOAEC is proposed as the study is not considered acceptable.
Reasons why the study report is not available for submission	The notifier has no access to this study report. The former RMS (BVL) has made the study report available to the current RMS.

B.6.3.3.7. Publications on repeated-dose toxicity via other routes – study 1

Data point:	CA 5.3.3/010
Report author	Mesnage, R. *et al.*
Report year	2018
Report title	Comparison of transcriptome responses to glyphosate, isoxaflutole, quizalofop-p-ethyl and mesotrione in the HepaRG cell line
Document No	doi.org/10.1016/j.toxrep.2018.08.005 E-ISSN: 2214-7500
Guidelines followed in study	Not applicable
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	**Conclusion GRG**: Yes/Reliable with restrictions **Conclusion AGG**: Reliable with restrictions

Full summary of the study according to OECD format

Together with three other herbicide active substances (quizalofop-p-ethyl, isoxaflutole and mesotrione) the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated.

Materials and methods
Chemicals - Glyphosate (purity ≥ 96 %) was purchased from Sigma-Aldrich, Gillingham, Dorset, UK.

HepaRG cell culture - Differentiated HepaRG™ cells (HPR 116) were purchased from Biopredic International (Rennes, France). Cells were thawed, suspended and plated in general purpose medium (Williams' E medium + GlutaMAX™) containing the ADD670 supplement. Cells were kept in general purpose medium until day 8, when the culture becomes well organized and includes well-delineated trabeculae and many canaliculi-like structures. At this time, the culture is composed of primitive biliary epithelial cells and mature hepatocytes with basal metabolic activities similar to freshly isolated primary cells. From day 8 to day 14, cells were switched to the test medium composed of Williams' E medium + GlutaMAX™ supplemented with 2 % foetal bovine serum and 1 % DMSO, as well as different concentrations of glyphosate or solvent as a control. Glyphosate was tested at 0.06 µM (concentration representative of low environmental exposure), 6 µM, and 600 µM.

Library generation and RNA-sequencing - A 100 ng aliquot of total RNA from each sample was used to prepare total RNA libraries using the KAPA Stranded RNA-Seq Kit with RiboErase, and samples were randomised before preparation. Polymerase chain reaction (PCR) was performed for 14 cycles for final library amplification. Resulting libraries were quantified using the Qubit 2.0 spectrophotometer and average fragment size assessed using the Agilent 2200 Tapestation. The transcriptome of HepaRG cells exposed to glyphosate was sequenced employing this strategy, except that the libraries were prepared as previously described. A total of 3 separate sequencing pools were created using equimolar quantities of each sample with compatible indexes: 2 with 17 samples each, and one with 16 samples. Paired-end reads of 75bp were generated for each library using the Illumina NextSeq®500 in con-junction with the NextSeq®500 v2 High-output 150-cycle kit.

Mass spectrometry-based metabolomics - Approximately 5,000,000 HepaRG cells per sample were harvested from the 6 well-plates to obtain a sufficient quantity of material to perform the metabolomics experiment. Cells were detached using 0.05 % trypsin EDTA, and centrifuged to eliminate trypsin residues. Finally, cell pellets were frozen at -80 °C pending analysis. Metabolomics analysis of the frozen cell pellets was conducted by Metabolon Inc. The sample extracts were stored overnight under nitrogen before preparation for analysis. The resulting extract was analysed on four independent instrument platforms: two different separate RP/UPLC-MS/MS with positive ion mode electrospray ionisation (ESI), a RP/UPLC-MS/MS with negative ion mode ESI, as well as by hydrophilic-interaction chromatography(HILIC)/UPLC-MS/MS with negative ion mode ESI as previously described. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Biochemical identifications are based on 3 criteria: retention index within a narrow retention time/index (RI) window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The use of all three data points can be utilised to distinguish and differentiate biochemicals. Peaks were quantified using the area-under-the-curve method.

Statistics - Metabolome data analysis was performed using in-house services of Metabolon Inc. Biochemical data were normalized with respect to protein concentration as determined by the Bradford assay. The Welch's two-sample t-test was used to test whether control and treatment group means are different from two independent populations. This version of the two-sample t-test allows for unequal variances. FDR methods and estimated q-values were used to account for the highest number of false positive results caused by the high number of statistical tests. The RNA-seq data analysis was performed using the new version of the Tuxedo protocol with HISAT2, StringTie and Ballgown. A standard linear model-based comparison of transcript abundance was performed without adjusting for other covariates to identify differentially expressed transcripts for each group. Although 3 concentrations were tested for glyphosate, no multigroup comparisons were used because dose spacing was too large to allow reliable conclusions to be drawn from these methods. Instead, pairwise comparisons were used.

Results

Control, untreated cell cultures presented no visible signs of aging after a 6-day exposure. Transcriptome profiles of HepaRG cells were then determined using the Illumina-based RNA sequencing platform. The highest concentration of glyphosate tested caused significant changes in transcriptome profiles. Alterations in gene expression caused by the 2 lowest concentrations (0.06 and 6 µM) failed to pass the statistical threshold that took into account the high number of tests performed. A total of 7 transcripts had their levels altered ($p < 0.05$) with glyphosate at 600 µM. The number of genes disturbed by the exposure to glyphosate was insufficient to use a functional annotation tool for pathway enrichment analysis. It is not clear if glyphosate lacks hepatic toxic effects at these concentrations or if this experimental design lacks sensitivity to detect hepatic effects of weak toxicants. To further explore changes in liver metabolism caused by glyphosate in greater detail, a global metabolome profiling of HepaRG cells exposed to three concentrations of glyphosate was explored. The Metabolon HD4 platform detected 802 named biochemicals in the HepaRG samples. Overall, glyphosate did not cause significant alterations in metabolome composition. However, exposure did cause a significant decrease in long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). HepaRG cells exposed to the lowest concentration of glyphosate tested (0.06 µM) showed the most dramatic effects in the levels of these fatty acids as either significant or trends towards significant lower levels. At the higher glyphosate concentrations of 6 µM and 600 µM, lower lipid levels were also observed but these did not reach statistical significance.

Discussion and conclusion

An in-depth investigation was conducted of transcriptome profile alterations in HepaRG human liver cells caused by exposure to pesticide active substances. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles. Interestingly, a follow-up metabolomics analysis of HepaRG cells treated with the lowest (0.06 µM) concentration of glyphosate revealed a significant decrease in the levels of LCFAs and PUFAs. Although these findings from an *in vitro* tissue culture model system cannot be readily translated to effects *in vivo*, they are nevertheless indicative of differences in toxicity potency between pesticide ingredients. The exact nature of this low dose effect of glyphosate cannot be determined from this single experiment, but it is possible that at higher concentrations, more overtly toxic mechanisms are masking the effects on lipids. Another possibility is that a saturation effect is occurring once the low dose is exceeded bearing in mind that glyphosate levels found in the HepaRG cells during the metabolomics analysis increased by 3.7-and 336.35-fold at the intermediate and highest concentrations tested compared to the negative controls. Glyphosate was the least toxic of the compounds tested in this study.

Assessment and conclusion by applicant:

Together with three other herbicide active substances (quizalofop-p-ethyl, isoxaflutole and mesotrione) the effect of glyphosate on the transcriptome and metabolome profile of differentiated HepaRG cells was investigated at 0.06, 6 and 600 µM. Glyphosate was found to be only weakly toxic inducing little change in transcriptome profiles when compared with the other herbicides tested. A follow-up metabolomics analysis of HepaRG cells exposed to glyphosate at 0.06 µM revealed a significant decrease in the levels of long chain fatty acids (LCFAs) and polyunsaturated fatty acids (PUFAs). At the higher glyphosate concentrations of 6 and 600 µM, lower lipid levels were also observed but these did not reach statistical significance. It is not clear, however, how these findings from an *in vitro* tissue culture model can be translated to effects *in vivo*.

Reliability criteria for *in vitro* toxicology studies by the applicant

Publication: Mesnage *et al*., 2018.	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	Glyphosate (purity ≥ 96 %). Source: Sigma-Aldrich, Gillingham, Dorset, UK.
Only glyphosate acid or one of its salts is the tested substance	N	Also three other pesticide active substances were tested (quizalofop-p-ethyl, isoxaflutole and mesotrione).
AMPA is the tested substance	N	
Test system clearly and completely described	Y	
Test conditions clearly and completely described	Y	
Metabolic activation system clearly and completely described	N	Not relevant.
Test concentrations in physiologically acceptable range (< 1 mM)	Y	0.06, 6 and 600 µM.
Cytotoxicity tests reported	N	
Transcriptomics and metabolomics methods described	Y	
Positive and negative controls	N	No positive controls included.
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		
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 no positive control was used and no cytotoxicity tests were performed to optimise the concentration range to be explored.		

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. However, no positive controls were included in the study. Overall, the study is considered reliable with restrictions (Klimisch score 2).

While the study gives some indication of a slight potential effect of glyphosate on transcriptome profile alterations in HepaRG human liver cells *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.6.3.3.8. Publications on repeated-dose toxicity via other routes – study 2

Data point:	CA 5.3.3/011
Report author	Kumar, S. *et al.*
Report year	2014
Report title	Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation
Document No	doi.org/10.1016/j.tox.2014.08.008 E-ISSN: 1879-3185
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	None
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion GRG: Yes/Reliable with restrictions Conclusion AGG: Unacceptable for the experiments with farm air / Reliable with restrictions (Klimisch score 2) for the other parts of the study

Full summary of the study according to OECD format

The aim of this study was to explore the mechanisms of glyphosate-induced pulmonary pathology by utilizing murine models and real environmental samples. C57BL/6, TLR4–/–, and IL-13–/– mice inhaled extracts of glyphosate-rich air samples collected on farms during spraying of herbicides or inhaled different doses of glyphosate and ovalbumin. The cellular response, humoral response, and lung function of exposed mice were evaluated. Exposure to glyphosate-rich air samples as well as glyphosate alone to the lungs increased: eosinophil and neutrophil counts, mast cell degranulation, and production of IL-33, TSLP, IL-13, and IL-5. In contrast, in vivo systemic IL-4 production was not increased. Co-administration of ovalbumin with glyphosate did not substantially change the inflammatory immune response. However, IL-13-deficiency resulted in diminished inflammatory response but did not have a significant effect on airway resistance upon methacholine challenge after 7 or 21 days of glyphosate exposure. Glyphosate-rich farm air samples as well as glyphosate alone were found to induce pulmonary IL-13-dependent inflammation and promote Th2 type cytokines, but not IL-4 for glyphosate alone.

Materials and methods

Mice

C57BL/6 female (6–9 weeks) mice were purchased from Jackson Laboratory (Sacramento, CA). TLR4–/– mice (backcrossed 10 generations) were received from Cincinnati Children's Hospital Medical Center (CCHMC). Both strains were subsequently bred in house. Female mice of wild type and IL-13–/– BALB/c background were received from the laboratory of Dr. Fred Finkelman, CCHMC. Mice were housed in individually ventilated cages in a pathogen free facility at the Department of Environmental Health, University of Cincinnati (UC) following the UC Institutional Animal Care and Use Committee (IACUC) guidelines and all experiments were conducted

following a UC IACUC – approved protocol.

Antibodies and reagents
The following antibodies for flow cytometry were purchased: Ly-6G (Gr-1) eFluor1 450 (RB6-8C5; Isotype Rat IgG2b) from eBioscience (San Diego, CA). CD16/CD32 (2.4G2; Isotype Rat IgG2b) and SiglecF-PE (E50-2440; Isotype Rat IgG2a) were purchased from BD PharMingen (San Jose, CA). A kit for measuring serum levels of MMCP1 was purchased from eBioscience.

Collection of farm air samples during summer pesticide spray seasons
Air samples were collected by three sets of total inhalable aerosol samplers (Button Inhalable Aerosol Sampler, SKC Inc., Eighty Four, PA) operated in parallel on three farms in Butler County, Ohio during summer glyphosate spray seasons. Samplers were installed at 1.5 m height at the edge of the field downwind from the spraying locations (sizes: approx. 5000 - 10,000 m^2). The sampling period was approximately 24 h starting from glyphosate spraying and air samples were collected at a flow rate of approximately 4 l/min on glass fiber filters. The filters from one set of samplers containing aerosolized glyphosate were eluted using PBS and the suspensions were filtered. A stock solution was prepared by pooling the samples collected from three farms (from now on referred as 'Real Env.') and used for intranasal treatment of mice. The filters from the other two sets of samplers were analysed for glyphosate and endotoxin to estimate the levels of glyphosate and endotoxin in 'Real Env.' samples.

Analysis of glyphosate in filter extracts
Glyphosate residues from filters were extracted using KH2PO4 buffer/1 M NaOH in an automatic shaker followed by freeze-drying. The freeze-dried samples were dissolved with deionised water and filtered through 0.45 mM Millipore filter. Glyphosate levels in the suspensions were determined by Abraxis ELISA Kit at 450 nm. The average amount of glyphosate per filter was 17.33 µg, which correspond to average airborne concentration of 22.59 ng/m^3.

Analysis of endotoxin in filter extracts
Endotoxin in filter extracts were analysed using the Limulus amebocyte lysate assay (Pyrochrome LAL; Associates of Cape Cod Inc., Falmouth, MA), as described previously. The samples were spiked with endotoxin standard of 0.50 EU/mL to assure that there was no inhibition or enhancement between the filter extracts and the reagents. The average amount of endotoxin per filter was 24.49 EU, which correspond to average airborne concentration of 4.87 EU/m^3.

Treatment of mice with farm-derived air samples, glyphosate and sensitization with OVA
PBS suspended farm air sample ('Real Env.'; estimated amount of glyphosate: 8.66 µg/mL) and reagent grade glyphosate (Sigma – Aldrich, St. Louis, MO) (100 ng, 1 µg or 100 µg) were delivered (in 30 µL) to the nose of anesthetised mice which were witnessed to aspirate the solution. Treatments were administered either: daily for 7 days or 3 times a week for 3 weeks. Same exposure schedule was followed for ovalbumin (OVA) alone (100 µg) and for OVA (100 µg) plus different dose of farm air sample and glyphosate. Mice were sacrificed 24 h after final airway treatment with sodium pentobarbital.

Histological analysis of lung
Formalin-fixed paraffin embedded lung sections (5 µm thick) were prepared for H&E and chloroacetate esterase (CAE) staining. The entire histological slide from each mouse was examined in blinded fashion and given a global categorical severity score based on infiltration of cells into parenchymal, peribronchial, and perivascular regions of lungs.

Immunohistochemical staining
To analyse IL-33 and TSLP expression in the lungs section, the following antibodies were used for immunostaining: mouse IL-33 (0.2 mg/mL, AF3626, R&D Systems, Minneapolis, MN); mouse TSLP biotinylated (0.2 mg/ml, BAF555, R&D Systems) and respective isotype controls (R&D Systems). IL-33 and TSLP antibody–antigen complex were detected using Cy3 donkey anti-goat IgG (1:10,000) (Invitrogen/Molecular probes, Grand Island, NY). Slides were counterstained with DAPI (Vector Labs, Burlingame, CA). Images were obtained using a Nikon A1R Si microscope.

Isolation of lung inflammatory cells
Lungs were perfused with PBS, removed, manually minced into 1–2 mm fragments and then placed in Hank's Balanced Salt Solution (Sigma–Aldrich) containing Liberase TL (50 µg/mL; Roche Diagnostics, Indianapolis, IN) and DNase I (0.5 mg/mL; Sigma – Aldrich). Tissue was digested at 37 °C in a CO_2 incubator for 30 min. The

tissue suspension was then passed through a 40 µm cell strainer. ACK lysis buffer (invitrogen) was used to clear red blood cells.

Flow cytometric analysis
Single cell suspensions from lungs (10^6 per mL) were blocked with anti-mouse CD16/CD32 antibodies before cell-surface staining. Cells were stained with fluorescently-labeled antibodies against SiglecF, Ly-6G/C (Gr-1), in different combinations according to the experiment. Analysis was performed using a FACSCanto II cytometer and FACSDIVA software (BD Biosciences). Eosinophils were defined as being SiglecF$^+$Gr-1$^+$ and neutrophils as SiglecF$^-$ Gr-1$^+$.

Cytokine measurement
IL-4, IL-10, IL-13, and IFN-γ production were measured by the in vivo cytokine capture assay (IVCCA). Briefly, biotinylated cytokine-specific mAbs were injected via tail vein immediately before the last airway treatment, and blood was collected 24 h later; sera or plasma were analysed with microtiter plates wells coated with corresponding anti-cytokine mAbs. Cytokine levels were also assessed in Bronchoalveolar lavage fluid (BALF) that was obtained 24 h after the last airway treatment. A kit for measuring in vivo IL-4 production by IVCCA, R46A2 and XMG1.2 anti-IFN-γ mAbs was purchased from Becton–Dickinson (Franklin Lakes, NJ); eBio1316H and eBio13A anti-IL-13 mAbs JES5-2A5 and JES5-16E3 anti-IL-10 mAbs ELISA Ready-SET-Go analysis kits for measurement IL-33 and IL-5 were purchased from eBioscience. Assays were performed according to the kit's manufacturer protocols.

Statistical analyses
Data were analysed with Sigma Plot 12.0 (Systat Software, Inc., San Jose, CA). Statistically significant differences in means were determined by one-way ANOVA followed by Bonferroni multiple comparison tests. Kruskal–Wallis tests were conducted if the data did not have a normal distribution. All the data are presented as mean ± SD for each group. Probability values of <0.05 were considered significant.

Results
Exposure of air samples collected during glyphosate spray on farms stimulates airway inflammation
Wild type C57BL/6 (WT) and TLR4–/– mice were intranasally exposed to 'Real Env.' samples (PBS suspended farm air samples) daily for 7 days. 'Real Env.' exposure was found to substantially increase the cell count in both the lungs and BAL fluid of WT and TLR4–/– mice. Additionally, the increase in pulmonary infiltrate in lungs was found to be higher in TLR4–/– than in WT mice (Fig. 1A and B). Similarly, an increase in eosinophil and neutrophil levels in 'Real Env.' treated mice (Fig. 1C–F) were observed. This inflammation was also confirmed by histological examinations (Fig. 1G) and elevated IgG1 and IgG2a levels. Additional experiments were conducted using reagent grade glyphosate of different doses. Administration of reagent grade glyphosate to the airway of mice produced substantial pulmonary inflammation whether the daily dose given was 100 ng, 1 µg or 100 µg for

7 days.

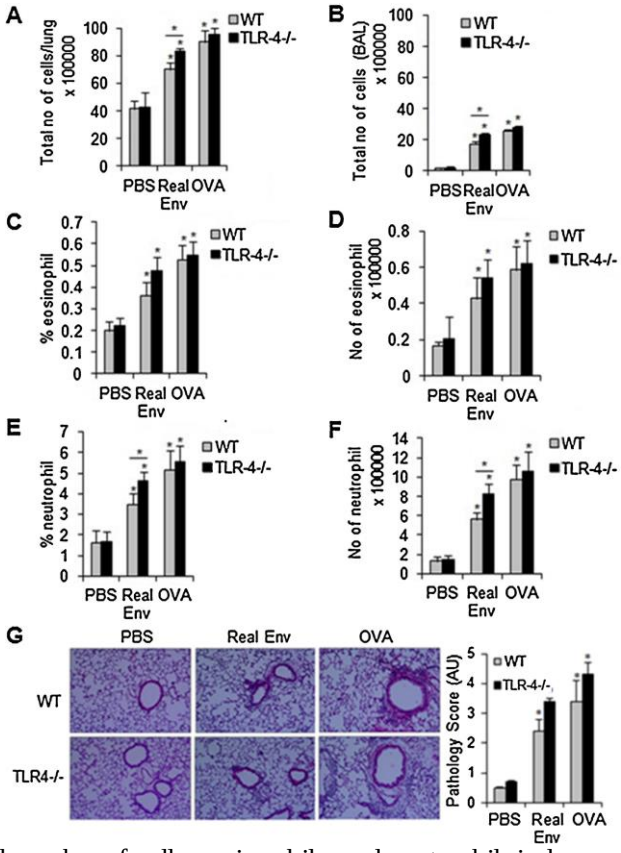

Figure 1: Increase in total number of cells, eosinophils, and neutrophils in lung and BAL fluids upon airway exposure to farm air samples ('Real Env.') and OVA for seven consecutive days (mean ± SD; $n = 8$). Increase in total number of cells in (A) lung and (B) BAL fluids. Increase in percentage (C) and total number (D) of eo sinophils and neutrophils (E, F) per lung upon exposure to farm air samples ('Real Env.'). (G) Representative lung sections (H&E staining) and its pathology score from mice treated with PBS, farm air samples ('Real Env.') and OVA intranasally for 7 consecutive days (mean ± SD; $n = 8$); magnification 200x. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS treated control and in between WT and TLR4 –/– mice group.

In the BALF and lung digests, a significant increase in the total cell count when treated with glyphosate at 1 µg or 100 µg (Fig. 2A and D) was found. Eosinophils (Fig. 2B and C), neutrophils, (Fig. 2E and F), and IgG1 and IgG2a levels) were also increased in glyphosate-treated mice compared to controls. However, No significant changes in the total cell count, eosinophils and neutrophils, IgG1 and IgG2a at glyphosate dose of 100 ng were found. Inflammation was confirmed by histological examination (Fig. 2M). Mice treated with both reagent grade glyphosate and OVA demonstrated significantly higher cell count (Fig. 2G and J), eosinophils (Fig. 2H and K), neutrophils (Fig. 2I and L), IgG1, and IgG2a compared to PBS treated mice.

Because pulmonary mastocytosis is typically observed in protein-allergen-induced experimental asthma, the

pulmonary mast cell burden in the mice were assessed.

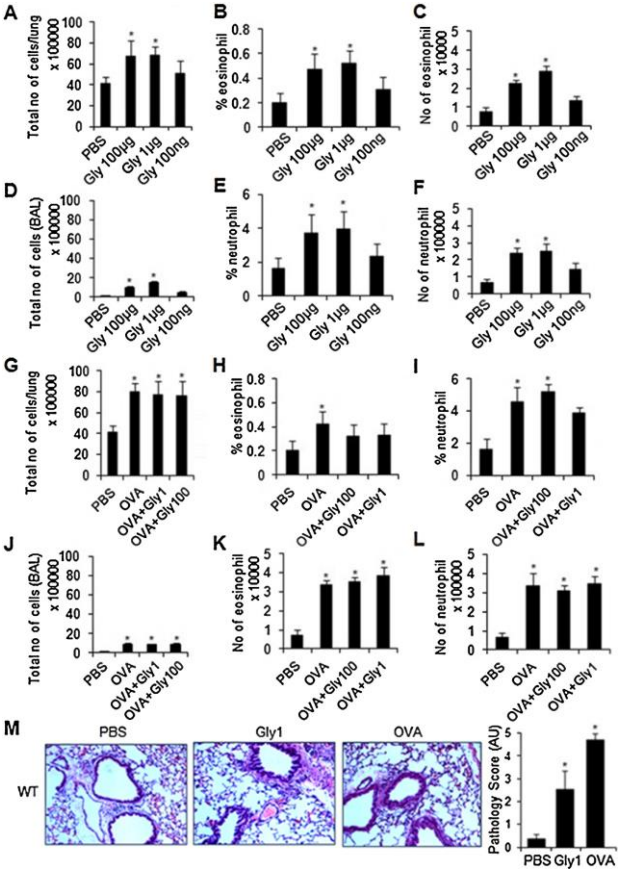

Figure 2: Increase in total number of cells, eosinophils, and neutrophils in lung and BAL fluids of WT mice upon airway exposure to glyphosate and combinations of glyphosate and OVA for seven consecutive days (mean ± SD; $n = 8$). Increase in total number of cells in (A) lungs and (D) BAL fluids upon exposure to different doses of glyphosate (100 ng, 1 µg or 100 µg). Increase in percentage (B) and total number (C) of eosinophils and neutrophils (E and F) per lung upon exposure to two doses of glyphosate. Increase in total number of cells in (G) lungs and (J) BAL fluids upon exposure to combination of glyphosate (1 µg or 100 µg) with OVA (100 µg). Increase in percentage and total number of (H and K) eosinophils and (I and L) neutrophils per lung upon exposure to OVA and combination of glyphosate, respectively. (M) Representative lung sections (H&E staining) and its pathology score from WT mice treated with PBS, glyphosate (1 µg) and OVA (100 µg) intranasally for 7 consecutive days (mean ± SD; $n = 8$); magnification 200 x. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS and treated WT mice group

A significant increase in mast cell number in lungs treated with the substances isolated from the air on active farms ('Real Env.') and reagent grade glyphosate (Fig. 3A and C) was not observed. However, the MCPT-1 levels were found to be substantially higher in both groups indicating increased mast cell degranulation in the treated mice

(Fig. 3B and D).

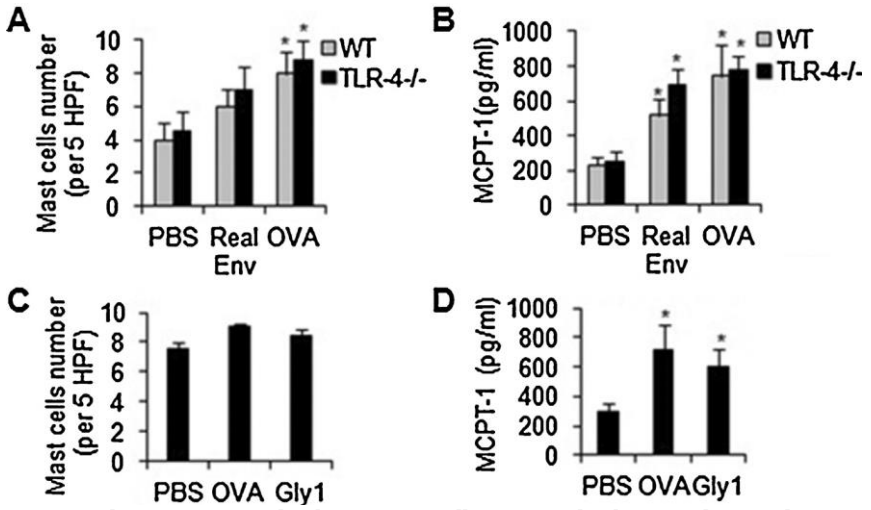

Figure 3: Farm air samples containing glyphosate as well as pure glyphosate alone induce increased mast cell degranulation but no increase in lung mast cell numbers upon airway exposure. (A) Mast cells number in CAE stained lung section and (B) serum MCPT-1 concentration in blood 4 h after last exposure of PBS, farm air samples ('Real Env.'), and ovalbumin (OVA). (C) Mast cells number in CAE stained lung section and (D) serum MCPT-1 concentration from mice treated with PBS, ovalbumin and 1 μg of glyphosate delivered to intranasally for 7 consecutive days (mean ± SD; n = 8). * Indicates statistically significant differences ($p < 0.05$) with respect to PBS and treated mice group.

Glyphosate-rich farm air samples induced airway inflammation and higher production of IL-10, IL-13, IL-5, IFN-γ and IL-4 but glyphosate alone failed to produce IL-4

To evaluate the glyphosate-induced inflammation, the systemic cytokine profile (Fig. 4A–E) of 'Real Env.' and glyphosate exposed mice were measured using IVCCA (Finkelman and Morris, 1999). Significantly higher levels of IL-5, IL-10, IL-13, and IL-4 were found upon treatment with 'Real Env.' alone in WT and TLR4 –/– mice (Fig. 4A–D) approaching the levels induced by treating with OVA alone. The production of IL-5, IL-13 and IL-10 following 'Real Env.' exposures was higher in TLR4–/– than in WT mice. No significant difference in IL-4 production between TLR4–/– and WT mice were found (Fig. 4D). Production of these cytokines in mice given two different doses of glyphosate were then tested and found significantly higher levels of IL-5, IL-10, IL-13 and IFN-γ (Fig. 4F) that approached those levels induced by treating with OVA alone. Notably, there was no additional or synergistic effect when OVA was co-administered with glyphosate (Fig. 4G). Another interesting finding is that glyphosate alone was unable to induce significant levels of IL-4 while airway treatment with glyphosate with OVA

did so.

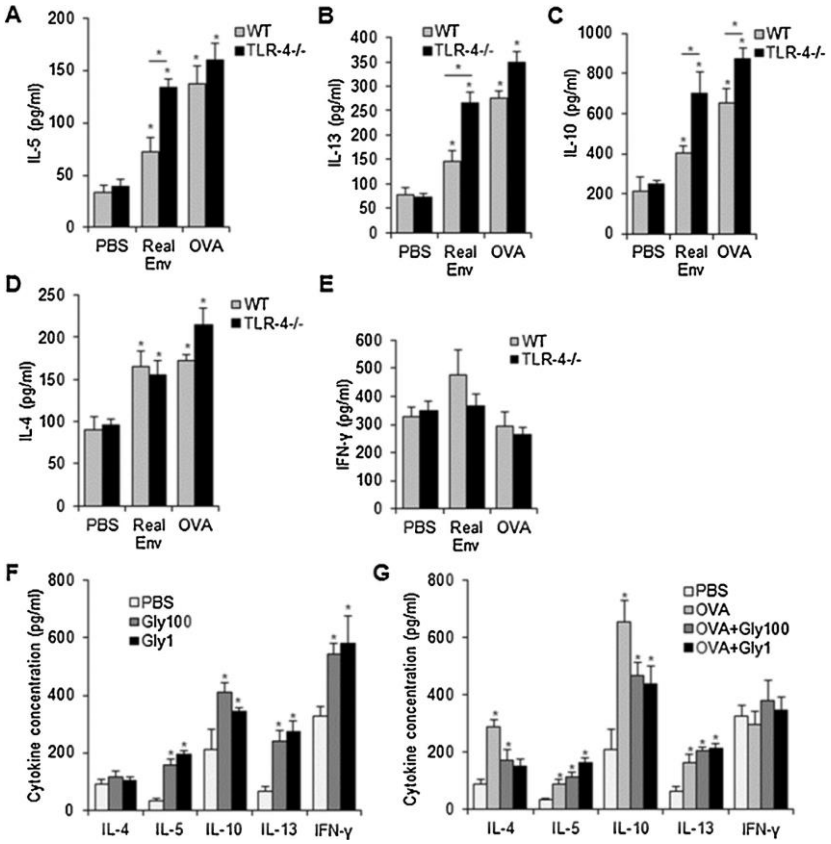

Figure 4: (A–E) Higher production of IL-5, IL-13, IL-10, IL-4 and no change in the IFN-γ levels upon exposure to farm air samples in WT and TLR4–/–mice. (F) The increased level of IL-5, IL-10, IL-13, IFN-γ and no change in the IL-4 levels upon glyphosate (1 or 100 µg) exposure to WT mice. (G) The increased level of IL-4, IL-5, IL-10, IL-13, and no change in IFN-γ levels upon combination of glyphosate (1 or 100 µg) and ovalbumin (100 µg) exposure to WT mice (mean ± SD; $n = 8$). Levels of cytokines were evaluated by IVCCA in serum of mice upon 7 consecutive days of intranasal treatment with farm air samples ('Real Env.') and glyphosate. Blood samples were collected 24 h after the last exposure. IL-5 was measured in the BAL fluids. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS treated control and in between WT and TLR4–/– mice group.

IL-33 and TSLP in lungs are increased upon exposure to glyphosate-rich air samples as well as reagent grade glyphosate alone

As the cytokine profile of mice treated with 'Real Env.' and glyphosate approximated those treated with OVA, mediators known to promote type 2 pathology were examined. IL-33 and TSLP appeared to be logical choices because of their well-recognized effector functions, and due to their source – the respiratory epithelium cells which would be the first cells to encounter inhaled glyphosate. The IL-33 and TSLP content of BALF were measured directly and found an abundance of both cytokines in 'Real Env.' – treated WT and TLR4–/– mice (Fig. 5A and B). IL-33 production was observed to be significantly higher in TLR4–/– mice compared to WT mice. An abundance of both cytokines in glyphosate-treated mice were observed (Fig. 5C and D). This finding was confirmed by immunohistochemical staining of IL-33 and TSLP in lung sections of glyphosate-treated mice (Fig. 5E) and 'Real Env.' – treated WT and TLR4–/– mice, which demonstrated substantial production of both

cytokines, which was limited to the respiratory epithelium after glyphosate exposure.

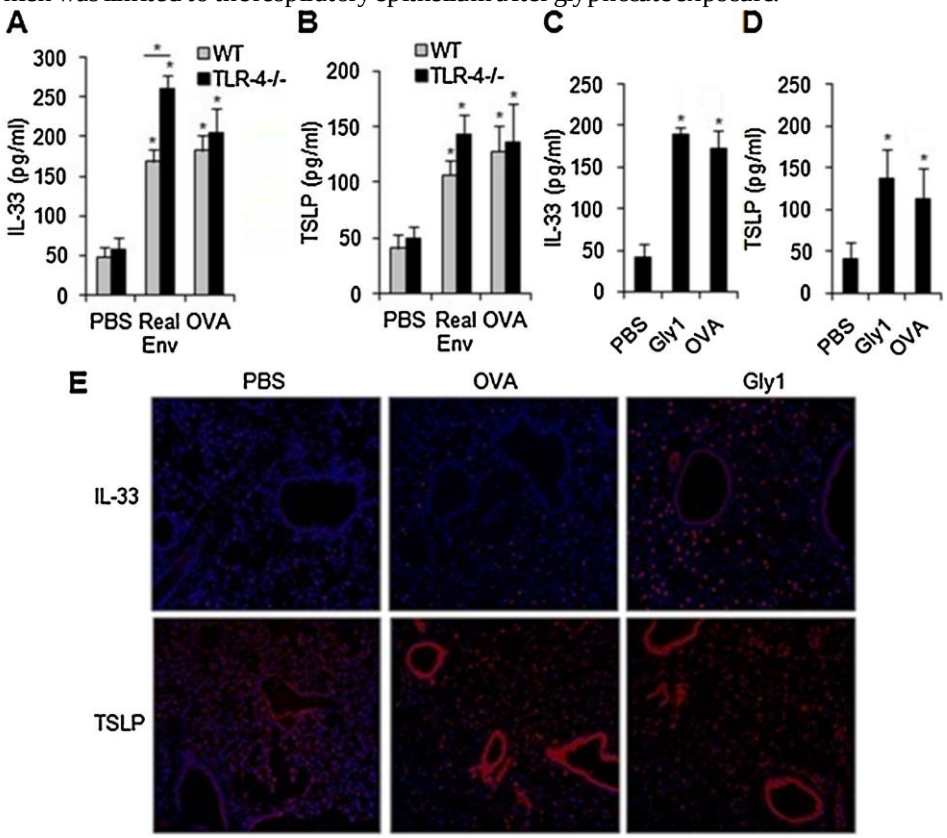

Figure 5: IL-33 and TSLP productions increased in the lung upon exposure to farm air samples and glyphosate. (A and B) ELISA based measurement of IL-33 and TSLP in BAL fluids of PBS, farm air samples and ovalbumin (100 µg) treated WT and TLR4–/– mice, respectively (mean ± SD; *n* = 8). (C and D) ELISA based measurement of IL-33 and TSLP in BAL fluids of PBS, OVA and pure glyphosate (1 µg) treated WT mice, respectively (mean ± SD; *n*=8). (E) Immunofluorescence staining of IL-33 and TSLP in the lung sections of the glyphosate treated WT mice, magnification 200x. * Indicates statistically significant differences ($p < 0.05$) with respect to PBS treated control and in between WT and TLR4–/– mice group.

Glyphosate-induced pulmonary inflammation is attenuated in IL-13 –/– mice

Glyphosate as a small molecule may not be efficiently presented to conventional T cells by antigen-presenting cells. The involvement of innate pathways upon glyphosate exposure, as hypothesized, was supported by the absence of an increased production of IL-4. This absence would have been expected if type 2 innate lymphoid cells (ILC2s) were the primary source of the IL-5 and IL-13 detected. IL-33 and TSLP have been well described to induce ILC2s, which in turn causes lung pathology particularly via IL-13-dependent mechanism. To test this hypothesis, IL-13 deficient mice were exposed to glyphosate for 7 and 21 days and assessed lung inflammation. While there was no change in IL-4 levels, the inability to produce IL-13 prevented the rise in IL-5 production, but not the rise in IL-10 production, at both time points during glyphosate treatment. Deficiency in IL-13 also prevented a significant rise in IL-33 and TSLP levels at the early time point but not the latter one (Fig. 6A–D). Lack of IL-13 production was also associated with significantly less ($p < 0.05$) severe cellular infiltration noted on histology (Fig. 6E). Despite significant inflammation, no airway hyper responsiveness was found in glyphosate-

treated wild type and IL-13–/– mice.

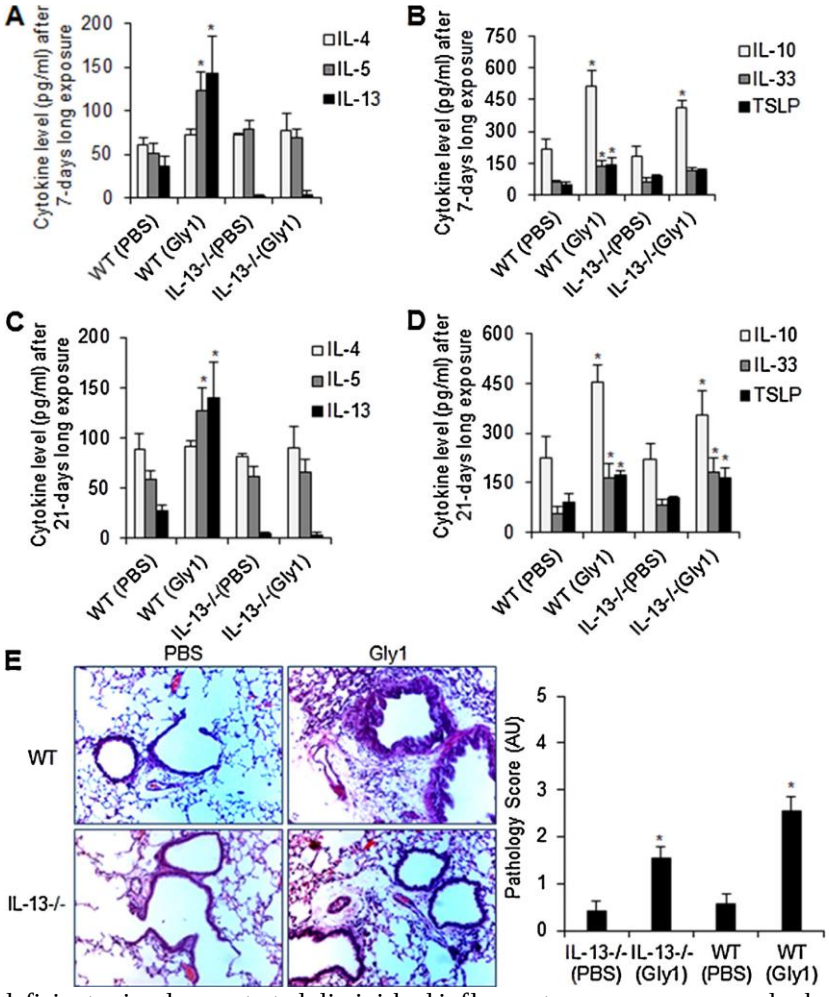

Figure 6: IL-13-deficient mice demonstrated diminished inflammatory response upon glyphosate exposure. (A, C) Diminished production of IL-5 but no change in IL-4 level, and (B, D) diminished production of TSLP, IL-33, IL-10 levels, between IL-13-deficient mice and WT mice upon glyphosate exposure (1 µg) for 7 or 21 days, respectively (mean ± SD; *n*=8). (E) Representative lung sections (H&E staining) from mice treated with PBS and glyphosate (1 µg) intranasally 3 times a week for 21 days; magnification 200 x (left panel). Arbitrary scores were based on inflammatory cells infiltration in lungs parenchyma, peribronchial, and perivascular regions. Analysis was performed in a double-blinded manner (right panel). * Indicates statistically significant differences (p <0.05) with respect to PBS treated control group.

Conclusion

The results demonstrate the capacity of glyphosate-rich air samples from farms as well as pure glyphosate to induce type 2 airway inflammation, over both short and longer time courses. Furthermore, glyphosate induced inflammation was found to be associated with induction of IL-33 and TSLP. This work also highlights the production of IL-13 as well as modulation of innate immune system by glyphosate, which may play an important role in exacerbation of airway inflammation by this low molecular weight chemical.

Assessment and conclusion by applicant:

This study evaluated nose-only exposure to glyphosate and collected farm air samples containing glyphosate and evaluated the immune response in the lungs. This is not a guideline study nor an endpoint used in risk assessment. This study in not usable for risk assessment in terms of hazard assessment. In terms of exposure, the study determined that average amount of glyphosate per filter from environmental samples after spray application to fields was 17.33 µg, which correspond to average airborne concentration of 22.59 ng/m^3. The method for the collection and analysis of the air samples was not validated and the assumptions and calculations used in the determination of the average airborne concentration were not provided, therefore the results cannot be verified. While the study itself is acceptable, it is unreliable in terms of usable endpoints for risk assessment.

Reliability criteria for *in vivo* toxicology studies by the applicant

Publication: Kumar et al., 2014.	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 is reagent grade. Source: Sigma - Aldrich, St. Louis, USA.
Only glyphosate acid or one of its salts is the tested substance	N	Sampling of aerosols from field spraying also contain co-formulants of the GBH applied.
AMPA is the tested substance	N	
Study		
Test species clearly and completely described	Y	Female mice of wild type and IL-13-/- BALB/c background.
Test conditions clearly and completely described	Y	
Route and mode of administration described	Y	Intranasal application of air samples taken during glyphosate field application (24 hours) and glyphosate.
Dose levels reported	Y	Field air sample and 100 ng, 1 µg or 100 µg of glyphosate delivered intranasally.
Number of animals used per dose level reported	Y	
Method of analysis described for analysis test media	Y	Glyphosate measured in air sample with ELISA kit.
Positive control	Y	Ovalbumin.
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	Y	For glyphosate.
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 the method for the collection and analysis of the air samples was not validated.		

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. However, as already stated by the applicant, the method for the collection of the air samples and the analysis of the glyphosate concentration in air samples was not validated. In addition, the RMS notes that the

analysis of endotoxin in filter extracts showed that the average amount of endotoxin per filter was 24.49 EU, which according to the authors corresponds to an average airborne concentration of 4.87 EU/m^3. As endotoxins itself may evoke an inflammatory response in lung cells, also a sample of farm air without glyphosate (e.g. air samples taken the day before spraying) should have been included in the experiments as a 'negative' control. In addition, besides endotoxins in farm air also other substances might be present in the air samples e.g. fine dust and other common air pollutants which might also lead to inflammatory reactions in the lung cells. Therefore, the experiments with farm air are not considered reliable by the RMS as it is not possible to ascribe the observed effects to glyphosate alone.
However, the parts of the experiments that were performed with reagent-grade glyphosate were considered as reliable with restrictions (Klimisch score 2).

Glyphosate exposure at 1 µg or 100 µg resulted in increased total cell count, eosinophils, neutrophils, and IgG1 and IgG2a levels in treated mice compared to controls. However, no effect was seen at 100 ng. Inflammation was confirmed by histological examination. But the study authors provided no information on which global categorical severity score was used for scoring the infiltration of cells into the lungs and the clinical relevance of the findings. Serum levels of MCPT-1 were higher after glyphosate treatment at 1 µg and comparable to OVA-treated mice, indicating increased mast cell degranulation in the OVA and glyphosate-treated mice. Further, IL-33 and TSLP were increased in the respiratory epithelium of glyphosate-treated mice compared with controls. It should be noted that the air samples and glyphosate (100 ng, 1 µg or 100 µg) were delivered (in 30 ml) to the nose of anesthetized mice in order to aspirate the solution. It is, however, unclear how aspiration relates to exposure to glyphosate after inhalation.
While this study provides some information on immune-responses occurring after glyphosate treatment in an experimental setting with laboratory mice, the study does not provide information that will directly impact the risk assessment of glyphosate.

B.6.4. GENOTOXICITY

Refer to separate RAR B.6.4

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

Refer to separate RAR B.6.5.

B.6.6. REPRODUCTIVE TOXICITY

Refer to separate RAR B.6.6.

B.6.7. NEUROTOXICTY

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.