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.6.1 - B.6.2 (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

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

B.6.1.1.1. Study 1

Data point	CA 5.1.1/001
Report author	[redacted]
Report year	2020
Report title	A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats
Report No	00050502
Document No	Not reported
Guidelines followed in study	None reported
Deviations from current test guideline	Only evaluation of plasmakinetics; clearance not calculated
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable (reliable without restrictions).

Executive summary

Glyphosate (97.7 % purity) was administered as dietary preparation for 14 consecutive days to four male and four female Sprague-Dawley rats to determine plasma toxicokinetic parameters of glyphosate and aminomethylphosphonic acid (AMPA). The target dose levels were 75 and 400 mg/kg bw/day in the low and high dose group, respectively. Thereafter, animals received basal diet for up to 3 days during which toxicokinetic samples were taken. Blood was sampled at 0.5, 2, 5, 8, 14, 24, 36 and 48 h after end of dietary administration.

All animals survived to the scheduled euthanasia. No abnormal clinical observations were noted and there were no differences in body weight or food consumption between test substance-treated groups.
The mean achieved dose levels were 72 and 385 mg/kg bw/day in the low and high dose group, respectively.

Systemic exposure to glyphosate and aminomethylphosphonic acid (AMPA) appeared to be independent of sex. Following 14 days of dietary administration of glyphosate, C_{max} and AUC_{0-48h} values for glyphosate increased with increasing dose in an approximately dose-proportional manner. Systemic exposure (AUC_{0-48h}) for AMPA was only noted at 385 mg/kg bw/day and was approximately 0.6 % of the systemic exposure (AUC_{0-48h}) for glyphosate following 14 days of dietary administration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate

Description: White powder

Lot/Batch #:	11493988
Purity:	97.7 %
Stability of test compound:	Expiry date 2020-04-01

2. Reference standards:

Identification:	Glyphosate
Lot/Batch #:	107671 (GLP-1507-24072-A)
Expiration date:	2021-08-31
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C and desiccated.
Identification:	Aminomethylphosphonic acid (AMPA)
Lot/Batch #:	107785 (GLP-1510-24158-A)
Expiration date:	2021-09-30
Storage Conditions:	Kept in a controlled temperature area set to maintain 21°C and desiccated.

3. Internal standards:

Identification:	Glyphosate-$^{13}C_3$,^{15}N
Lot/Batch #:	107369 (GLP-0904-19814-A)
Expiration date:	2021-04-30
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.
Identification:	AMPA-^{13}C,^{15}N,D_2
Lot/Batch #:	107360 (GLP-1002-20244-A)
Expiration date:	2020-01-31
Storage Conditions:	Kept in a refrigerator set to maintain 4°C.

4. Vehicle and/ or positive control: PMI Nutrition International, LLC Lab Diet Certified Rodent Diet 5002

5. Test animals:

Species:	Rat
Strain:	Sprague-Dawley; Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 8 weeks
Sex:	Males and females
Weight at dosing:	272 – 331 g (males), 190 – 238 g (females)
Acclimation period:	At least 14 days
Diet/Food:	PMI Nutrition International, LLC Lab Diet Certified Rodent Diet 5002, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in polycarbonate, solid-bottom cages containing appropriate bedding material.
Environmental conditions:	Temperature: 20 - 26°C Humidity: 30 - 70 % Air changes: not reported 12-hour light/dark cycle (except during designated procedures)

B. STUDY DESIGN

In life dates: 2019-07-08 to 2019-08-22

Animal assignment and treatment

Eight rats (4 male, 4 female) received repeated oral target doses of either 75 (Group 1) or 400 mg/kg bw/day (Group 2) by dietary administration for 14 consecutive days. Thereafter, animals received basal diet for up to 3 days during which toxicokinetic samples were taken.
Observations for mortality were made at least twice daily (once daily on days of receipt and necropsy), beginning upon arrival through termination/release. Cage side observations were made at least once daily beginning on Day 0 except for the days of detailed clinical observations. Detailed clinical observations were made within 4 days of receipt, on day of randomization, Day 0, weekly (± 2 days) thereafter and on the day of scheduled euthanasia.
Individual body weights were recorded within 4 days of receipt, on day of randomization, Days 0, 3, 7, 14 and on the day of scheduled euthanasia. Food consumption was examined on Days 0, 7, and 14.
Blood samples (0.5 mL/time point) were taken from the tail vein into heparinised tubes from each group at 0.5, 2, 5, 8, 14, 24, 36 and 48 h on Study Day 14. Each group was sacrificed upon completion of the specified sampling schedule.

Food evaluation

Homogeneity and concentration of the test material in the dietary preparations were assessed via HPLC. The initial concentrations of test substance in the diet were based on historical data for this age, strain, and sex of animal and were corrected for purity. Concentrations were adjusted weekly based on the expected average body weight and food consumption in order to meet target dose levels for each treatment group. The mean amounts of glyphosate consumed (mg/kg bw/day) by each sex per dose group were calculated from the mean food consumed (g/kg bw/day) and the appropriate target concentration of test substance in the food (mg/kg bw/day). Food efficiency (body weight gained as a percentage of food consumed) was calculated and reported.

Sample Processing

Blood samples were mixed gently centrifuged. The resultant plasma was separated, transferred to duplicate uniquely labelled polypropylene tubes, kept on ice packs until verified, and then transferred on dry ice to storage. Samples were stored in a freezer set to maintain a target of -70°C.

Measurement of glyphosate and aminomethylphosphonic acid (AMPA)

Bioanalytical samples were analysed for concentration of glyphosate and aminomethylphosphonic acid (AMPA) using a validated LC-MS/MS method (Nethero, Method No. 50.501A.RP).
Study design parameters were as following:

Parameters	
Analytes	Glyphosate Aminomethylphosphonic acid (AMPA)
Internal Standards (ISs)	Glyphosate-$^{13}C_3$,^{15}N AMPA-^{13}C,^{15}N,D_2
Species / Matrix	Rat plasma
Anticoagulant	K_2EDTA
Assay Volume	0.05 mL
Extraction Procedure	Protein filtration and solid phase extraction (SPE)
Instrumentation	UHPLC-MS/MS (Sciex API 5500)
Detection	Electrospray ionization (negative-ion mode)
Regression, Weighting	Quadratic, $1/x^2$
Calibration Curve Concentrations	10.0 to 5000 ng/mL

In addition to the experimental samples, each analytical run consisted of a minimum of duplicate calibration standards at nine concentrations, at least one blank matrix sample, at least one blank matrix with internal standard (IS) sample, and at least duplicate quality control (QC) samples at a minimum of three concentrations.

The acceptance criteria described in SOP BAC-008 were required to be met for an analytical run to be accepted. The back-calculated concentrations of the calibration samples should be within ± 15 % (20 % at the lower limit of quantitation (LLOQ)) of the nominal value and at least 75 % of the calibration samples, with a minimum of 6 calibration sample levels to fulfil these criteria.

The calculated concentration of at least two-thirds of the QC samples must be within ± 15 % of their nominal concentrations. At least half of the replicates at each QC concentration must be within ± 15 % of the nominal concentration.

Concentrations less than the LLOQ (<10 ng/mL for glyphosate and AMPA) were set to 0 for toxicokinetic analysis.

Calculations

For each dose group, maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (T_{max}) and area under the plasma concentration-time curve (AUC) were calculated.

The AUC from time 0 - 48 h ($AUC_{0\text{-}48h}$), the AUC from time 0 - 24 h ($AUC_{0\text{-}24h}$), and the AUC from time 0 to the time of the final quantifiable sample (AUC_{Tlast}) were calculated by the linear trapezoidal method for all dose groups with at least 3 consecutive quantifiable concentrations. For Day 14, C_{min} was used as an estimate of the 0-hour concentration.

Half-life values ($T_{1/2}$) were reported for composite plasma concentration-time profiles with sufficient plasma concentrations in the terminal elimination phase (at least 3 samples not including T_{max}) and an adjusted R^2 of ≥0.9. The female to male exposure ratio (F:M) and the AMPA:Glyphosate ratio were calculated for each dose group using the following formulas:

$$F{:}M = \frac{AUC_{0-48h\ Female}}{AUC_{0-48h\ Male}}$$

$$AMPA{:}Glyphosate = \frac{AUC_{0-48h\ AMPA}}{AUC_{0-48h\ Glyphosate}}$$

When T_{last} did not equal the last collection interval, the percent of AUC extrapolated (% AUC_{Extrap}) for $AUC_{0\text{-}24h}$ or $AUC_{0\text{-}48h}$ was calculated as:

$$\%\ AUC_{Extrap} = \frac{AUC_{0-24h} - AUC_{Tlast}}{AUC_{0-24h}} \times 100$$

$$\%\ AUC_{Extrap} = \frac{AUC_{0-48h} - AUC_{Tlast}}{AUC_{0-48h}} \times 100$$

All $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}48h}$ values were calculated with less than 25 % extrapolation. The % AUC_{Extrap} data are not reported but are maintained in the study file.

Data exclusion and additional information

All AMPA plasma concentrations were below the LOQ (<10 ng/mL) for Group 1 (72 mg/kg bw/day) and therefore were excluded from the toxicokinetic data analysis. No other data exclusions were performed for toxicokinetic data analysis.

There was insufficient volume for analysis of glyphosate and AMPA from the sample obtained from one male dosed at 385 mg/kg bw/day glyphosate (Animal no. 6140) on Day 14 at the 5 h collection time point.
$AUC_{0\text{-}24h}$ values were reported for informational purposes only to reflect the daily glyphosate dosing interval;

however, were not discussed.

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals survived until scheduled euthanasia.

B. CLINICAL OBSERVATIONS

There were no abnormal clinical observations noted during the study.

C. BODY WEIGHT

There were no differences in body weights or body weight gains between treated groups.

D. FOOD AND TEST SUBSTANCE CONSUMPTION DATA

From Day 0 to Day 14, food consumption and food efficiency were similar between the 75 and 400 mg/kg bw/day groups.

The average achieved glyphosate dose was determined using the food consumption data from Days 0 to 14 and resulted in 74 and 389 mg/kg bw/day for 75 and 400 mg/kg bw/day group males, and 71 and 382 mg/kg bw/day for 75 and 400 mg/kg bw/day group females. The average test substance consumption for combined sexes amounted to 72 and 385 mg/kg bw/day for the 75 and 400 mg/kg bw/day group, respectively (please refer to the table below). These average achieved glyphosate doses were used for the toxicokinetic evaluations.

Table 6.1.1.1-1: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats [redacted] 2020): Mean (± SD) food consumption data (mg/animal/day)

a.i. Dose level	75 mg/kg bw/day		400 mg/kg bw/day	
Day	**Males**	**Females**	**Males**	**Females**
0 - 7	22 ± 2.0	16 ± 2.0	22 ± 2.3	16 ± 1.2
7 - 14	23 ± 1.5	16 ± 1.8	22 ± 1.9	16 ± 1.7
0 - 14	23 ± 1.7	16 ± 1.9	22 ± 2.1	16 ± 1.4

Table 6.1.1.1-2: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats [redacted] 2020): Average test substance consumption data (mg/kg bw/day)

Target dose levels [mg/kg bw/day]	Average achieved dose levels [mg/kg bw/day]	
	Males	Females
75	74	71
400	389	382

E. TOXICOKINETIC EVALUATIONS

Systemic exposure to glyphosate and AMPA appeared to be independent of sex. There were no consistent differences in individual plasma concentrations, C_{max}, and AUC_{0-48h} values between males and females (female to male AUC_{0-48h} ratios ranged from 0.784 to 0.867). Please refer to tables below. Therefore, the following discussion is based on the data for males and females combined.

Glyphosate

The variability in mean glyphosate plasma concentrations, as measured by coefficient of variation (CV) values, ranged from 10.3 - 54.5 % following 14 days of dietary administration of 72 and 385 mg/kg bw/day glyphosate.

Glyphosate was quantifiable up to 48 h and peak glyphosate plasma concentrations were observed at 0.5 h following 14 days of dietary administration of 72 and 385 mg/kg bw/day glyphosate. The mean plasma concentration declined exponentially in male and female rats irrespective of the dose level (based on a coefficient of determination (R^2) between 0.974 and 0.997 calculated for males and females at high and low dose for the logarithmic regression).

Following 14 days of dietary administration of glyphosate, C_{max} and AUC_{0-48h} values for glyphosate were dose dependently increased at the high dose. A 5.3-fold increase in glyphosate dose resulted in an approximate 6.8-fold increase in glyphosate C_{max} values and an approximate 5.4-fold increase in glyphosate AUC_{0-48h} values.

The half-lives ($T_{1/2}$) for glyphosate were 11.0 and 13.0 h at 72 and 385 mg/kg bw/day, respectively.

AMPA

AMPA concentrations were all below the limit of quantitation (BQL) for the 72 mg/kg bw/day glyphosate dose level. The variability in mean AMPA plasma concentrations, as measured by CV values, ranged from 17.4 - 84.3 % following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. The higher variability observed (84.3 %) was the result of BQL values converted to 0 for parameter estimates and averaged with quantifiable results. The variability in mean AMPA plasma concentrations without this value ranged from 17.4 - 27.5 %.

AMPA concentrations were below the limit of quantitation within 24 h but was detected up to 14 h after termination of treatment. Peak AMPA plasma concentrations were observed at 0.5 h following 14 days of dietary administration of 385 mg/kg bw/day glyphosate.

C_{max} and AUC_{0-48h} values for AMPA were 39.2 ng/mL and 302 h × ng/mL, respectively.

Systemic exposure (AUC_{0-48h}) for AMPA was less than the systemic exposure of glyphosate following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. The AMPA:Glyphosate ratio, based on AUC_{0-48h}, was 0.00596 (i.e. 0.6 %). The $T_{1/2}$ value for AMPA was 7.32 h at 385 mg/kg bw/day.

Table 6.1.1.1-3: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats [redacted] 2020): Mean (± SD) glyphosate and AMPA plasma concentrations (ng/mL) in male and female rats after dietary administration of glyphosate at 72 and 385 mg/kg bw/day for 14 days

Time Point	72 mg/kg bw/day		385 mg/kg bw/day	
	Male	Female	Male	Female

[h]	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
	Plasma concentrations in ng/mL							
0.5	841±145	BQL[1]	636±74.0	BQL	5310±521	40.6±13.1	4690±294	37.8±8.48
2	674±232	BQL	523±134	BQL	4280±320	28.8±3.68	3510±472	24.3±4.73
5	599±79.9	BQL	520±73.5	BQL	3500±230	23.0±3.73	2560±500	18.2±6.31
8	438±155	BQL	307±125	BQL	2240±411	16.2±5.81	2040±509	16.9±3.81
14	282±36.3	BQL	272±37.5	BQL	1520±135	9.65±6.66	1030±300	7.28±8.41
24	115±53.2	BQL	66.1±35.7	BQL	491±100	0.00[2]±N/A	385±87.9	0.00±N/A
36	51.9±18.2	BQL	41.4±12.1	BQL	255±94.2	0.00±N/A	176±32.5	0.00±N/A
48	22.5±10.3	BQL	17.2±6.86	BQL	134±31.3	0.00±N/A	110±12.2	0.00±N/A

N/A not applicable
BQL below the limit of quantitation
[1]All AMPA plasma concentrations were below the limit of quantitation for Group 1 (72 mg/kg bw/day) and therefore excluded from the toxicokinetic data analysis.
[2]All concentrations less than the lower limit of quantitation (LLOQ <10.0 ng/mL for glyphosate and AMPA) were set to 0 for toxicokinetic analysis.

Table 6.1.1.1-4: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats [redacted] 2020): Toxicokinetic parameters in plasma after dietary administration of glyphosate (72 mg/kg bw/day) for 14 days

Parameter		Male		Female		Combined	
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
C_{max}	[ng/mL]	841	N/A	636	N/A	738	N/A
C_{max}/dose	[kg×ng/mL/mg]	11.7	N/A	8.83	N/A	10.3	N/A
T_{max}	[h]	0.5	N/A	0.5	N/A	0.5	N/A
T_{last}	[h]	48	N/A	48	N/A	48	N/A
AUC_{Tlast}	[h×ng/mL]	10400	N/A	8260	N/A	9330	N/A
$AUC_{0\text{-}24h}$	[h×ng/mL]	8950	N/A	7270	N/A	8110	N/A
$AUC_{0\text{-}24h}$/dose	[h×kg×ng/mL/mg]	124	N/A	101	N/A	113	N/A
$AUC_{0\text{-}48h}$	[h×ng/mL]	10400	N/A	8260	N/A	9330	N/A
$AUC_{0\text{-}48h}$/dose	[h×kg×ng/mL/mg]	144	N/A	115	N/A	130	N/A
F:M[a)]	-	N/A	N/A	0.795	N/A	N/A	N/A
AMPA:Glyphosate[b)]	-	N/A	N/A	N/A	N/A	N/A	N/A
$T_{1/2}$	[h]	10.2	N/A	10.2	N/A	11.0	N/A

N/A not applicable
a) $F:M = \frac{AUC_{0-48h\ Female}}{AUC_{0-48h\ Male}}$
b) $AMPA:Glyphosate = \frac{AUC_{0-48h\ AMPA}}{AUC_{0-48h\ Glyphosate}}$

Table 6.1.1.1-5: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats [redacted] 2020): Toxicokinetic parameters in plasma after dietary administration of glyphosate (385 mg/kg bw/day) for 14 days

Parameter		Male		Female		Combined	
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
C_{max}	[ng/mL]	5310	40.6	4690	37.8	5000	39.2
C_{max}/dose	[kg×ng/mL/mg]	13.8	N/A	12.2	N/A	13.0	N/A
T_{max}	[h]	0.5	0.5	0.5	0.5	0.5	0.5
T_{last}	[h]	48	14	48	14	48	14
AUC_{Tlast}	[h×ng/mL]	57000	276	44700	245	50700	260
$AUC_{0\text{-}24h}$	[h×ng/mL]	50200	325	39600	281	44700	302
$AUC_{0\text{-}24h}$/dose	[h×kg×ng/mL/mg]	130	N/A	103	N/A	116	N/A
$AUC_{0\text{-}48h}$	[h×ng/mL]	57000	325	44700	281	50700	302

Table 6.1.1.1-5: A GLP 14-Day Oral (Dietary) Toxicokinetic Study of Glyphosate in Sprague Dawley Rats [redacted] 2020): Toxicokinetic parameters in plasma after dietary administration of glyphosate (385 mg/kg bw/day) for 14 days

Parameter		Male		Female		Combined	
		Glyphosate	AMPA	Glyphosate	AMPA	Glyphosate	AMPA
AUC_{0-48h}/dose	[h×kg×ng/mL/mg]	148	N/A	116	N/A	132	N/A
F:M[a)]	-	N/A	N/A	0.784	0.867	N/A	N/A
AMPA:Glyphosate[b)]	-	N/A	0.00569	N/A	0.00629	N/A	0.00596
$T_{1/2}$	[h]	12.8	7.48	8.86	7.04	13.0	7.32

N/A not applicable

a) $F:M = \frac{AUC_{0-48h\ Female}}{AUC_{0-48h\ Male}}$

b) $AMPA:Glyphosate = \frac{AUC_{0-48h\ AMPA}}{AUC_{0-48h\ Glyphosate}}$

III. CONCLUSIONS

Systemic exposure to glyphosate and AMPA after dietary application appeared to be independent of sex. Following 14 days of dietary administration of glyphosate, C_{max} and AUC_{0-48h} values for glyphosate increased with increasing dose in an approximately dose proportional manner.
Systemic exposure (AUC_{0-48h}) for AMPA was approximately 0.6 % of the systemic exposure (AUC_{0-48h}) for glyphosate following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. AMPA was not detected in plasma samples from rats treated with 72 mg/kg bw/day.

Assessment and conclusion by applicant:

The aim of the study was to evaluate plasma kinetic parameters for two days following repeated dietary administration of glyphosate at target concentrations of 75 and 400 mg/kg bw/day for 14 consecutive days.

The average test substance consumption for combined sexes amounted to 72 and 385 mg/kg bw/day.
Systemic exposure to glyphosate and AMPA appeared to be independent of sex indicated by the calculated male/female AUC_{0-48h} ratios of 0.8 for glyphosate at both dose levels and the male/female AUC_{0-48h} ratios of 0.9 for AMPA at the high dose. No AMPA was detected in plasma at the low dose. Following 14 days of dietary administration of glyphosate, C_{max} and AUC_{0-48h} values for glyphosate were dose-dependendly increased at the high dose. The plasma concentration of AMPA was low, i.e. approximately 0.6 % of the systemic exposure (AUC_{0-48h}) for glyphosate following 14 days of dietary administration of 385 mg/kg bw/day glyphosate. Plasma elimination of glyphosate was fast with half-life values of 11.0 and 13.0 h at 72 and 385 mg/kg bw/day, respectively. Plasma elimination of AMPA was fast with a half-life of 7.3 h.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. The internal exposure of the compound is dose dependent (C_{max}, AUC) while the plasma elimination rate is fast and independent of dose. At the low dose tested no AMPA was detected in the plasma and in high dose animals the AMPA:Glyphosate ratio is 0.006. The results were independent of sex.

B.6.1.1.2. Study 2

Data point	CA 5.1.1/002
Report author	[redacted]
Report year	1996
Report title	[^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat
Report No	1413/2-1011

Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1995)
Deviations from current test guideline	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 to be acceptable.

Executive summary

[^{14}C]-labelled glyphosate was administered by gavage, as a single dose of 1 or 100 mg/kg bw to fasted male and female Sprague-Dawley rats to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C]-concentrations. The biliary excretion was measured directly *via* bile duct cannulation. The proportion and ratio of metabolites were also investigated.

Absorption from the gut was slow and limited. Mean maximum plasma concentrations of 0.016 µg equiv./mL (male) and 0.0362 µg equiv./mL (female) were observed between 1.5 and 6 h (males, mean 3.9 h) and 4 and 12 h (females, mean 8 h) after dosing at a nominal dose level of 1 mg/kg bw. Following oral administration at a nominal dose level of 100 mg/kg bw maximum plasma levels were 8.909 µg equiv./mL (male) and 7.634 µg equiv./mL (female) at about 4 h following administration. A comparison of the AUC_{0-24} values shows an approximate 100-fold increase with a corresponding increase in dose, indicating that absorption is independent of the dose level. The distribution of [^{14}C]-glyphosate was rapid and widespread. Maximum tissue concentrations were observed between 4 and 12 h post-dose (1 mg/kg bw) and about 6 h post-dose (100 mg/kg bw). Examination of tissue levels indicated that [^{14}C]-glyphosate was not retained in the tissues with the exception of less than 1 % in bone at 72 h post-dose.

Metabolite profiles of pooled urine and faecal samples were investigated by HPLC analysis. Only one major peak was detected in urine and faeces (>90 % of total activity) which was subsequently identified as glyphosate by LC-MS/MS in representative samples. A minor component was observed in the radiochromatogram, which had a similar retention time to AMPA, however, due to the very low levels this could not be definitively identified. Within 168 h the mean total recovery was 98.31 % (male) and 98.81 % (female) at a nominal dose level of 1 mg/kg bw, with 72.62 % (male) and 62.39 % (female) being recovered in faeces and 18.44 % (male) and 27.15 % (female) being detected in urine. At the high dose level (100 mg/kg bw) elimination *via* urine (39.42 % male; 43.08 % female) was quantitatively more significant than for the low dose group. Faecal elimination accounted for 41.23 % (male) and 42.38 % (female). Negligible amounts of radioactivity were detected in the bile (<0.08 µg equiv./g) at the dose level of 1 mg/kg bw, thus indicating that radioactivity recovered in faeces is most likely unabsorbed material. The increase in renal elimination following administration at 100 mg/kg bw suggest an increase in absorption. However, a high inter-animal variation is also observed which prevents a firm conclusion being drawn. Furthermore, the pharmacokinetic data indicates that absorption is linear which contradicts the suggestion of increased absorption. The highly ionisable nature of the test substance could be a contributory factor to the variability in the degree of absorption.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification: Glyphosate

Description: Not reported

Lot/Batch #:	08808TG and H95D161A
Purity:	96 % and 95.3 %, respectively
Stability of test compound:	Not reported
2. Radiolabelled test material:	
Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	24, Lot 3 and 25, Lot 4-7
Radiochemical purity:	>99 % (HPLC and TLC)
Specific activity:	310 µCi/mg, 53 mCi/mmol
Stability of test compound:	Stable over 24 h under the conditions of the study
3. Reference substance:	
Identification:	Aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9)
Description:	Not reported
Lot/Batch #:	50526010
Purity:	Not reported
Stability of test compound:	Not reported
4. Vehicle and/ or positive control:	Deionised water
5. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	6 - 10 weeks
Sex:	Males and females
Weight at dosing:	179 - 280 g (males) and 167 - 205 g (females)
Acclimation period:	Approximately 1 week
Diet/Food:	SQC Rat and Mouse Maintenance Diet No. 1, Expanded (Special Diet Services, Stepfield, Witham, Essex, UK), *ad libitum* Diet was removed the evening before and returned 4 h after administration
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of 5 per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing wood saw dust After dosing: Excretion-balance experiments - individually in glass metabolism cages Blood/plasma kinetics - in wire floor cages Tissue distribution - in wire floor cages
Environmental conditions:	Temperature: 21 ± 2°C (24 and 26°C on two consecutive days) This deviation did not affect the study outcome Humidity: 40 - 70 % Air changes: not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported, according to QA statement procedure took place in December 1995

Animal assignment and treatment: Preliminary excretion study

Four fasted rats (2 males, 2 females) received single oral doses of 100 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0 - 12, 12 - 24, and every 24 h for 7 days in receivers cooled with solid CO_2. Faeces were collected every 24 h for 7 days. Expired air was passed through duplicate traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed 12, 24, 48 and 72 h after dosing. The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 10 fasted rats (5 males, 5 females) received single oral doses of either 1 or 100 mg/kg bw by gavage. Blood samples (0.1 mL) were taken from the tail vein into heparinised tubes at the following times from each animal:

Prior to administration and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after administration.
Blood was centrifuged to separate plasma and radioactivity was determined in plasma.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 24 fasted rats (12 male, 12 female) received single oral doses of either 1 or 100 mg/kg bw by gavage. The animals were divided into four groups of six (3 per sex) and sacrificed 4, 12, 24 and 72 (low dose) or 4, 6, 24 and 72 h (high dose) after dosing. Animals were exsanguinated under halothane anaesthesia. Following tissues/organs were taken or sampled for radioactivity measurements:

Adrenals, bone, brain, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, muscle (quadriceps), gonads, plasma, skin, spleen, and residual carcass.

The contents of the gastrointestinal tract were analysed separately.

Animal assignment and treatment: Biliary excretion study

16 rats were cannulated (8 males, 8 females), of which 14 received single oral doses of 1 mg/kg bw/day by gavage. Following incision animals were placed in metabolism cages and allowed to recover for 24 h. Fully recovered animals were dosed after they were fasted overnight, and bile, urine and faeces were taken from the animals at the following times:

Bile: Prior to administration, 0 - 1, 1 - 4, 4 - 6, 6 - 12, 12 - 24 and 24 - 48 h p.a.
Urine, faeces: 0 - 24 and 24 - 48 h p.a. in vessels cooled with solid CO_2.

The interiors of the cages were rinsed with water after each collection time. At the end of the collection period cages were rinsed with water and methanol. Samples were analysed accordingly.

Measurement of radioactivity

Pooled faecal samples were extracted with water prior to solid phase extraction. Urine samples were diluted with water prior to solid phase extraction. Solid phase extraction was performed using columns, conditioned with methanol and de-ionised water. The samples were loaded onto the cartridge washed with de-ionised water, methanol and again water. Radioactivity was eluted using formic acid (5 % v/v). The eluate was freeze dried and reconstituted in water prior to HPLC analysis and where appropriate LC-MS (samples of 100 mg/kg bw dose group).

A suitable volume of solubilising agent was added to tissue samples. After an appropriate incubation time, liquid scintillant was added and samples were subjected to liquid scintillation counting (LSC). Samples of faecal residues, cage debris homogenates, blood and bone were combusted, absorbed, mixed with scintillation cocktail and analysed by LSC thereafter. Combustion and trapping efficiencies were found to be in excess of 96 % and all reported data are, therefore, uncorrected. Radioassays were performed in duplicates.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats of the excretion studies were pooled and analysed directly by HPLC. Representative samples were then submitted for analysis by mass spectrometry. The samples were analysed for the presence of glyphosate and the potential metabolite aminomethyl phosphonic acid (AMPA). Following samples were pooled and analysed for each dose group and sex:

Excretion study: Urine 12 - 24 h, Faeces 24 - 48 h
Biliary excretion study: Urine 24 - 48 h, Faeces 24 - 48 h
High performance liquid chromatography (HPLC)

The gradient elution method was used for sample analysis (column: Sperisorp SAX 250 x 4.6 mm id, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35). The system was linked to a radio-detector. Following HPLC analysis, representative samples were submitted for analysis by mass spectroscopy (samples of 100 mg/kg bw dose group).

Liquid chromatography - Mass Spectrometry (LC - MS)

A VG Quattro triple quadrupole mass spectrometer with electrospray LCMS interface connected to a Jasco ternary gradient HPLC system and a Lablogic β-Ram radio detector were used.

Mode: positive ion electrospray
Scan range: m/z 50 – 250
Mobile phases: water or 1 M formic acid

Glyphosate was detected using Multiple Reaction Monitoring (MRM) of m/z 170 → 88. AMPA was detected using Selected Ion Recording (SIR) of m/z 112.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

In a preliminary study with a single dose of 100 mg/kg bw (two rats/sex) the mean total recovery of radioactivity within 7 days was 100.3 % (male) and 95.15 % (female). No relevant radioactivity could be detected in expired air or carcass.

The initial observation was confirmed in the main study with 10 rats per dose. Please refer to table given below.

Mean total recovery of radioactivity in rats receiving a single dose of 1 mg/kg bw was 98.31 % in males and 98.81 % in females. Elimination of radioactivity was almost complete within the first 48 h after dosing. The major route of elimination after oral dosing was faeces with 72.62 % and 62.39 % recovered in males and females, respectively, with most of the radioactivity being excreted within the first 24 h after dosing, suggesting this proportion of the dose was not systemically absorbed. During the 7 days observation period 24.92 % (male) and 34.86 % (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose.

After administration of 100 mg/kg bw to rats mean total recovery of radioactivity was 96.31 % in males and 98.50 % in females. Elimination of radioactivity in the urine (including cage wash 53.27 % in males and 55.04 % in females) was quantitatively more significant compared to the low dose group. Faecal elimination accounted for 41.23 % in males and 42.37 % in females. Again most of the radioactivity was recovered within the first 48 h after dosing.

Table 6.1.1.2-1: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted], 1996): Excretion balance (in mean % of applied dose) at 168 h post dosing

Balance/Excretion	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Urine 0 - 12	9.52	15.47	31.30	34.93
Urine 12 - 24	6.14	7.59	4.68	4.46
Urine 24 - 48	2.10	3.03	2.40	2.32
Urine 48 - 72	0.35	0.56	0.46	0.71
Urine 72 - 96	0.15	0.20	0.27	0.33
Urine 96 - 120	0.09	0.14	0.14	0.15
Urine 120 - 144	0.06	0.10	0.10	0.10
Urine 144 - 168	0.04	0.06	0.08	0.07
Cage wash	6.48	7.71	13.85	11.96
Subtotal urine + cage wash	**24.92**	**34.86**	**53.27**	**55.04**
Faeces 0 - 24	63.93	49.69	30.46	32.28
Faeces 24 - 48	7.21	10.93	9.96	4.46
Faeces 48 - 72	0.65	1.46	0.55	1.10
Faeces 72 - 96	0.09	0.16	0.12	4.42
Faeces 96 - 120	0.03	0.06	0.06	0.06
Faeces 120 - 144	ND	0.07	0.06	0.04
Faeces 144 - 168	0.71	0.02	0.03	0.01
Subtotal faeces	**72.62**	**62.39**	**41.23**	**42.37**
Cage debris	0.03	0.58	0.98	0.10
Carcass	0.75	0.98	0.84	0.98
Total	**98.31**	**98.81**	**96.31**	**98.50**

B. BILIARY EXCRETION OF RADIOACTIVITY

Biliary excretion was determined in biliary cannulated rats receiving 1 mg/kg bw. Within 48 h 94.63 % and 95.99 % of radioactivity were recovered in males and females, respectively. Major route of elimination was faeces. Negligible amounts of radioactivity were detected in the bile, providing strong evidence that low doses of systemic glyphosate are eliminated almost exclusively in the urine. Please refer to table given below.

Table 6.1.1.2-2: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted] 1996): Excretion balance (in mean % of applied dose) at 48 h post dosing in biliary excretion study

Balance/Excretion	1 mg/kg bw	
	Males	Females
Urine	27.45	24.21
Faeces	55.33	60.97
Bile	**0.031**	**0.076**
Cage wash	6.571	6.769
Cage debris	0.262	0.146
Carcass	4.989	3.817
Total	**94.63**	**95.99**

C. CONCENTRATION OF RADIOACTIVITY IN THE PLASMA

Following a single oral dose of 1 mg/kg bw of the test substance low levels of radioactivity were detected in plasma. Concentrations of radioactivity declined rapidly such that the levels of radioactivity were below the detection limit in most animals by 24 h. The mean terminal elimination half-lives were 10.86 h and 8.07 h with corresponding AUC of 0.319 and 0.340 µg equiv./mL*h in males and females, respectively. As the elimination half-lives could not be calculated for several animals of the high dose group, mean AUC_{0-24} (0.257 and 0.338 µg equiv./mL*h in males and females, respectively) were calculated to compare the results of both groups.

Following a single oral dose of 100 mg/kg bw of the test substance mean maximal plasma concentration of 8.91 (male) and 7.63 µg equiv./mL (female) were observed 2 - 4 h post-dose in males and 4 h post dose in females. Mean AUC_{0-24} were 58.2 and 50.7 µg equiv./mL*h in males and females, respectively. Levels of radioactivity were below the detection limit in males by 48 h and in females by 72 h. Please refer to table given below.

Table 6.1.1.2-3: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] 1996): Kinetic parameters in plasma after single oral dose of 1 or 100 mg/kg bw (n=5)

	1 mg/kg bw		100 mg/kg bw	
	Males	**Females**	**Males**	**Females**
C_{max} (µg equiv./mL)	0.016	0.037	8.909	7.634
T_{max} (h)	3.900	8.000	3.600	4.000
AUC_{0-24} (µg equiv./mL*h)	0.257	0.338	58.200	50.700
AUC (µg equiv./mL*h)	0.319	0.340	[1]	[1]
Terminal half life (h)	10.860	8.065	[1]	[1]

[1] Could not be calculated

D. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

After administration of 1 mg/kg bw [^{14}C]-labelled glyphosate radioactivity was detected in all tissues, except brain within 4 hours (see Table 6.1.1.2-1). Apart from the gastrointestinal tract (and content) and carcass, the kidney and bone were the only tissue with a notable content of radioactivity throughout the observation period ranging from 0.012 - 0.463 µg equiv/g and 0.062 - 0.201 µg equiv/g, respectively. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity (see Table 6.1.1.2-3). After administration of 100 mg/kg bw was detected in all tissues within 4 h (see Table 6.1.1.2-2). Again, apart from the gastrointestinal tract and carcass the kidney and bone were the only tissue with a notable content of radioactivity throughout the observation period ranging from 1.19 - 132.2 µg equiv/g and 10.42 - 35.45 µg equiv/g, respectively. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity (see

Table **6.1.1.2-4**).

Table 6.1.1.2-1: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] 1996): Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	0.014	0.024	0.020	0.009	0.023	0.031	0.022	0.009
Blood	0.010	0.015	0.001	0.002	0.020	0.009	0.002	<0.001
Bone	0.062	0.105	0.201	0.123	0.091	0.140	0.134	0.112
Brain	<0.001	0.003	0.003	0.002	0.002	0.001	0.002	0.002
Carcass	0.021	0.028	0.049	0.016	0.035	0.076	0.045	0.024
Fat	0.022	0.005	0.003	0.002	0.013	0.010	0.006	0.002
GIT + contents	13.040	1.333	1.272	0.026	11.630	3.531	1.314	0.075
Heart	0.006	0.004	0.003	0.002	0.010	0.006	0.004	0.001
Kidney	0.463	0.380	0.307	0.020	0.424	0.387	0.129	0.012
Liver	0.012	0.013	0.022	0.012	0.016	0.018	0.015	0.012
Lung	0.009	0.009	0.013	0.006	0.019	0.013	0.009	0.006
Muscle	0.003	0.001	0.002	<0.001	0.006	0.003	0.002	0.001
Ovaries	-	-	-	-	0.031	0.018	0.021	0.007
Plasma	0.017	0.011	0.006	<0.001	0.027	0.015	0.004	<0.001
Skin	0.010	0.026	0.016	0.006	0.029	0.016	0.106	0.014
Spleen	0.004	0.009	0.010	0.005	0.010	0.009	0.010	0.005
Testes	0.004	0.002	0.001	0.001	-	-	-	-

Table 6.1.1.2-2: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] 1996): Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	2.936	5.610	1.856	0.338	8.161	7.244	1.522	0.504
Blood	4.545	4.900	0.016	ND	5.719	1.923	0.218	ND
Bone	24.660	31.360	18.600	11.140	35.450	24.420	17.010	10.420
Brain	0.344	0.699	0.269	0.221	0.619	0.630	0.293	0.215
Carcass	6.097	26.530	4.978	1.843	10.910	38.410	7.206	3.057
Fat	1.366	1.547	0.290	0.120	3.826	2.042	0.393	0.115
GIT+contents	1155.000	544.600	47.750	1.279	1057.000	401.800	59.580	4.320
Heart	2.063	3.424	0.363	0.140	3.704	2.282	0.314	0.092
Kidneys	105.500	127.700	17.440	1.433	132.200	55.770	10.800	1.191
Liver	2.942	4.970	1.831	1.165	5.105	5.564	1.552	0.981
Lung	3.495	4.206	1.069	0.423	6.476	4.623	0.999	0.443
Muscle	0.827	0.887	0.168	0.026	1.698	1.141	0.213	0.051
Ovaries	-	-	-	-	7.532	5.407	1.260	0.438
Plasma	6.479	5.406	0.359	ND	10.830	3.033	0.403	ND

Table 6.1.1.2-2: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] 1996): Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean µg equiv./g)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Skin	2.884	3.520	1.293	0.313	6.106	22.480	1.543	0.435
Spleen	1.277	2.678	0.974	0.479	2.337	1.237	0.937	0.395
Testes	0.949	0.942	0.203	0.104	-	-	-	-

ND not detected

Table 6.1.1.2-3: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat ([redacted] 1996): Radioactivity in tissues after single oral dose of 1 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
Adrenals	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Brain	<0.001	0.003	0.002	0.002	0.002	0.001	0.002	0.002
Carcass	1.236	1.668	3.048	1.045	1.887	4.115	2.542	1.405
GIT + contents	94.310	17.670	12.990	0.342	89.940	41.740	13.760	0.910
Heart	0.002	0.001	0.001	0.001	0.005	0.003	0.002	0.001
Kidney	0.392	0.304	0.255	0.016	0.348	0.341	0.110	0.011
Liver	0.040	0.050	0.113	0.059	0.055	0.070	0.073	0.057
Lung	0.005	0.005	0.007	0.003	0.012	0.008	0.005	0.004
Ovaries	-	-	-	-	0.002	0.001	0.001	<0.001
Spleen	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.001
Testes	0.004	0.002	0.002	0.001	-	-	-	-

Table 6.1.1.2-4: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted], 1996): Radioactivity in tissues after single oral dose of 100 mg/kg bw (in mean % of applied dose)

Tissue	Males				Females			
	4 h	6 h	24 h	72 h	4 h	6 h	24 h	72 h
Adrenals	0.001	0.001	<0.001	<0.001	0.002	0.001	<0.001	<0.001
Brain	0.003	0.005	0.002	0.002	0.006	0.006	0.003	0.002
Carcass	4.620	8.549	2.402	1.014	8.288	5.879	2.752	1.254
GIT + contents	85.430	64.870	5.456	0.199	75.050	48.910	7.509	0.676
Heart	0.009	0.015	0.001	0.001	0.016	0.009	0.001	<0.001
Kidney	0.870	1.109	0.151	0.012	1.165	0.535	0.096	0.011
Liver	0.104	0.180	0.110	0.060	0.183	0.214	0.088	0.050
Lung	0.027	0.024	0.006	0.003	0.040	0.027	0.007	0.003
Ovaries	-	-	-	-	0.004	0.002	0.001	<0.001
Spleen	0.003	0.007	0.002	0.001	0.006	0.003	0.003	0.001
Testes	0.011	0.010	0.003	0.001	-	-	-	-

E. METABOLITE PROFILING

After analysis of the pooled samples by HPLC, comparison of chromatograms indicated that the metabolism of the compound was not influenced by the sex or dose level. The peak with the majority of radioactivity could be allocated to [^{14}C]-glyphosate standard. A peak with <1 % of the total radioactivity was thought to correspond to AMPA. The presence of glyphosate could be confirmed by mass spectroscopy, whereas the presence of AMPA could not be verified due to technical problems.

F. RECOVERY OF RADIOACTIVITY

The mean total recovery of radioactivity within 168 h was about 94 - 99 % at the low dose and the high dose.

III. CONCLUSIONS

In conclusion, there was no apparent sex difference in the absorption, metabolism, distribution and excretion of [^{14}C]-glyphosate following oral administration at both dose levels. A time-dependent decrease of radioactivity was observed for all investigated tissues, which indicates that accumulation rather does not occur.

After oral administration of glyphosate absorption, distribution, metabolism and excretion were independent of dose level and sex. Absorption was limited and distribution was rapid and extensive. Metabolism was negligible. Elimination was essentially complete within 48 h, with the majority of radioactivity recovered in faeces, likely being the unabsorbed dose. The remaining radioactivity was excreted with the urine.

Assessment and conclusion by applicant:

After single oral administration of 10 or 100 mg/kg bw glyphosate to both male and female rats at least about 25 % are absorbed (based on the amount excreted *via* urine including cage wash). About 62 - 73 % (low dose) and 41 - 42 % (high dose) of the dose are excreted *via* faeces and 25 - 35 % (low dose) and 53 - 55 % (high dose) urine including cage wash, respectively, mainly as the unchanged parent compound within 7 days after administration. The presence of aminomethyl phosphonic acid (AMPA) could not be verified due to technical issues.
Biliary excretion experiments confirmed that bioavailability is rather low and can be calculated from the amount recovered in urine and cage wash. Furthermore, bioavailable glyphosate is excreted completely within 48 h, whereby almost no biotransformation occurs.

Investigation of pharmacokinetics in plasma revealed that plasma peak levels were reached 4 h (males) and 8 h (females) after administration of the low dose and 4 h (males and females) after administration of the high dose. No further significant gender-dependent differences were noted.

Determination of concentrations in tissue revealed overall low levels at all investigated sampling time points and dose levels, except the GI tract and contents. For all other investigated tissues and organs the levels were each below or equal to 0.39 % and 1.17 % (both determined for kidney) of the administered low and high dose, respectively. Radioactivity levels decreased time-dependently or reached the plateau levels to <2 %. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Urinary excretion of the compound is higher in high dose animals, which is not seen in similar studies (CA 5.1.1/004). Taking into account the negligible biliary excretion, oral absorption is likely represented by renal elimination. There are no notable differences in male and female animals.
The highest concentrations of glyphosate residues were found in the bone at 72 hours post dosing, which suggest slower elimination from this tissue.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.3. Study 3

Data point	CA 5.1.1/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat
Report No	[redacted] P/4940
Document No	Not reported
Guidelines followed in study	MAFF (Japan) Metabolism Study (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	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 to be acceptable.

Executive summary

A single oral dose of 10 mg/kg bw of [14C]-labelled glyphosate acid (glyphosate, >98 % radiochemical purity) was administered as aqueous solution by gavage to Alpk:APfSD rats (5 per sex) to determine tissue distribution, excretion of [14C] and plasma [14C] concentrations 72 h post administration.

Excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in the faeces, within 24 h. Males excreted means of 13.3 % and 88.5 % of the dose in urine and faeces, respectively, over 72 h. Females excreted means of 11.1 % and 88.7 % of the dose in urine and faeces, respectively, over the same period. At the end of the observation period (72 h) a low percentage of radioactivity was present in all tissues examined, with highest concentrations found in the bone and intestinal tract plus contents.

In conclusion, after oral application glyphosate acid was excreted rapidly and extensively, predominantly in faeces.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-phosphonomethyl glycine)
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 %
Stability of test compound:	Stable throughout the experiment

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>98 %
Specific activity:	1.580 GBq/mMol
Stability of test compound:	Stable throughout the experiment
3. Vehicle and/ or positive control:	Deionised water
4. Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Male/female
Weight at dosing:	195 - 235 g
Acclimation period:	At least 5 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Excretion study and quantitative tissue distribution

Ten rats (5 male, 5 female) received a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw (10 mL/kg, 0.6 MBq/kg of dosing solution) by gavage and were placed back in glass metabolism cages immediately thereafter. Urine was collected at 0 - 6, 6 - 12, 12 - 24, 24 - 36, 36 - 48 and 48 - 72 h hafter dosing in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24, 24 - 36, 36 - 48 h and 48 - 72 h intervals. The interior of the cages were washed with water after each collection time. At the end of the study, cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20°C until analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. One blood sample was retained and divided into two portions samples were collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, gonads, heart, kidneys, liver, lungs, muscle (femoral), spleen, salivary glands and residual carcass.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcasses were than solubilised in Soluene-350® whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for [^{14}C]-radioactivity to a 1 % standard deviation of the count or for a maximum of 10 min, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (means of 77.8 % in males, and 80.7 % in females). In the urine, means of 11.5 % and 9.4 % of the radioactivity were eliminated in the first 24 h in male and female rats respectively. Within the observation period of 72 h means of 101.8 % (male) and 99.6 % (female) of the administered radioactivity were excreted. There were no differences in the cumulative excretion patterns between the sexes. Please refer to table given below.

Table 6.1.1.3-5: Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted] 1996): Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	10 mg/kg bw (oral gavage)	
	Males	Females
Urine 0 - 6	3.7	3.5
Urine 6 - 12	4.5	3.3
Urine 12 - 24	3.3	2.6

Table 6.1.1.3-5: Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted] 1996): Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	10 mg/kg bw (oral gavage)	
	Males	Females
Urine 24 - 36	0.8	0.7
Urine 36 - 48	0.4	0.4
Urine 48 - 72	0.3	0.2
Cage wash	0.3	0.4
Subtotal urine + cage wash*	**13.3**	**11.1**
Faeces 0 - 12	42.3	48.1
Faeces 12 - 24	35.5	32.6
Faeces 24 - 36	6.6	3.9
Faeces 36 - 48	2.8	2.9
Faeces 48 - 72	1.3	1.2
Subtotal faeces*	**88.5**	**88.7**
Total*	**101.8**	**99.6**

* Minor numerical deviations may occur due to rounding

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 0.51 µg equiv./g (male) and 0.40 µg equiv./g (female), followed by the gastrointestinal tract (GIT) plus contents with 0.15 µg equiv./g (males and females). Lower mean concentrations between 0.01 and 0.07 µg equiv./g were found in kidneys, liver, lungs, spleen, salivary glands and ovaries. Mean concentrations of approximately 0.06 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of either sex. All other concentrations were either similar to or lower than the corresponding blood concentrations. The mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was 0.6 % for males and 0.5 % for females. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes. Please refer to table given below.

Table 6.1.1.3-6: Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted] 1996): Radioactivity in tissues after a single oral dose of 10 mg/kg bw at 72 h

Tissue	Males		Females	
	% of dose	µg equiv./g	% of dose	µg equiv./g
Blood	N/A	0.011	N/A	0.009
Bone (femur)	N/A	0.511	N/A	0.395
Brain	0.001	0.011	0.001	0.009
Fat (abdominal)	N/A	0.007	N/A	<0.004
Heart	<0.001	0.012	<0.001	0.011
Kidneys	0.007	0.068	0.004	0.049
Liver	0.036	0.059	0.022	0.044
Lungs	0.002	0.031	0.001	0.026
Muscle (femoral)	N/A	0.007	N/A	0.006
Ovary	-	-	<0.001	0.024
Plasma	N/A	N/A	N/A	<0.004
Residual Carcass	0.542	0.062	0.458	0.056
Salivary glands	<0.001	0.017	<0.001	0.018
Spleen	0.001	0.026	0.001	0.024
Testes	0.001	0.007	-	-
Total	**0.590**	**N/A**	**0.488**	**N/A**
GIT plus contents	0.186	0.152	0.172	0.152

N/A not applicable

C. RECOVERY OF RADIOACTIVITY

The total mean recovery, including excreta, tissues and residual carcass, was 102.6 % for male and 100.3 % for female rats.

III. CONCLUSIONS

After a single oral dose, glyphosate acid was excreted rapidly and predominantly in faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone having the highest tissue radioactivity.

Assessment and conclusion by applicant:

After single oral administration of 10 mg/kg bw glyphosate to male and female rats, at least 11 % was absorbed (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 89 % of the dose are excreted *via* faeces, whereby again main portions are excreted within 24 h. No gender difference was noted.

Determination of concentrations in tissue 72 h after application revealed overall low levels each below or equal to 0.6 % of the dose (residual carcass) or 0.51 µg equiv./g (bone) for both genders. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Excretion essentially complete at 72 hours post dosing and most of it is excreted within the first 24 hours.
Again, the highest concentrations of glyphosate residues were found in the bone at 72 hours post dosing, suggesting affinity of the compound to bone.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.4. Study 4

Data point	CA 5.1.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat
Report No	[redacted] P/4942
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	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 to be acceptable.

Executive summary

[^{14}C]-labelled glyphosate acid (glyphosate, >98 % radiochemical purity) was administered by gavage, as a single dose of 1000 mg/kg bw to non-fasted Alpk:AP$_f$SD rats (5 per sex) to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C] concentrations 72 h post administration.

Excretion of radioactivity was rapid for rats of both sexes and most administered dose was eliminated, principally in faeces, within 24 h. Males excreted means of 16.7 % and 89.6 % of the dose in urine and faeces respectively over 72 h. Females excreted means of 17.5 % and 84.5 % of the dose in urine and faeces respectively over the same period. The rates of excretion were thus similar for both sexes. Upon termination of the experiment (72 h) the mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was 0.5 % for males and 0.6 % for females.

In conclusion, there was no apparent sex difference in distribution and excretion of [^{14}C]-glyphosate acid following oral administration at 1000 mg/kg bw. Glyphosate acid was excreted rapidly and extensively, predominantly in the faeces.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-phosphonomethyl glycine)
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 %
Stability of test compound:	Stable throughout the experiment

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>98 %
Specific activity:	1.580 GBq/mMol
Stability of test compound:	Stable throughout the experiment

3. Vehicle and/ or positive control: Deionised water

4. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Male/female
Weight at dosing:	182 - 235 g
Acclimation period:	At least 4 days
Diet/Food:	PCD rat diet (SDS Ltd. Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*

Housing:	During acclimatisation: Groups of 6 per cage and sex, in stock rat cages After dosing: Excretion-balance experiments - individually in metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Excretion study and quantitative tissue distribution

Ten non-fasted rats (5 male, 5 female) received single oral doses of 1000 mg [^{14}C]-glyphosate acid/kg bw (10 mL/kg, 6 MBq/kg) by gavage and were placed in stainless steel metabolism cages immediately thereafter. Urine was collected at 0 - 6, 6 - 12, 12 - 24, 24 - 36, 36 - 48 and 48 - 72 h after dosing in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24, 24 - 36, 36 - 48 h and 48 - 72 h intervals. The interior of the cages were washed with water after each collection time. At the end of the study, cages were washed with ethanol/water 1:1 (v/v). Samples were stored at -20°C until analysis.
Animals were exsanguinated by cardiac puncture under halothane anaesthesia. One blood sample separated into two portions was collected in heparinised vials. One was centrifuged to separate plasma. Following tissues/organs were taken or sampled for radioactivity measurements: Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, gonads, heart, kidneys, liver, lungs, muscle (femoral), spleen, salivary glands and residual carcass.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcasses were and gastrointestinal tract (GIT) plus contents were homogenised. Liver and fat were than solubilised in Soluene-350®, whereas GIT and residual carcass were oxidised. All other tissues were solubilised without prior homogenisation.

Sample oxidation

Samples were oxidised in a Packard Tricarb sample oxidiser. The [^{14}C]-carbon dioxide generated was absorbed into Carbo-sorb E® and mixed with Permafluor E+® scintillant prior to analysis by LSC.

Liquid scintillation counting (LSC)

Samples and dilutions of the dosing preparation were mixed with Optiphase Hi-Safe 3® and counted for [^{14}C]-radioactivity to a 1 % standard deviation of the count or a maximum of 10 min in Packard Tricarb instruments, whichever occurred first. The results obtained were corrected for background activity and counting efficiency using [^{135}Ba] as the external source.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After a single oral dose to rats, excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (78.7 % in males, and 71.3 % in females). In the urine, means of 15.3 % and 16.0 % of the radioactivity were eliminated in the first 24 h in males and females, respectively. Within the observation period of 72 h, means of 106.4 % (male) and 102.3 % (female) of the administered radioactivity were excreted. There were no differences in the cumulative excretion patterns between the sexes. Please refer to table given below.

Table 6.1.1.4-7: Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat ([redacted] 1996): Excretion balance (in mean % of applied dose) at 72 h post dosing

Balance/Excretion	1000 mg/kg bw	
	Males	**Females**
Urine 0 - 6	7.9	9.7
Urine 6 - 12	5.0	3.9
Urine 12 - 24	2.5	2.4
Urine 24 - 36	0.7	0.8
Urine 36 - 48	0.4	0.5
Urine 48 - 72	0.3	0.3
Cage wash	0.1	0.2
Subtotal urine + cage wash*	**16.9**	**17.8**
Faeces 0 - 12	36.4	19.7
Faeces 12 - 24	42.2	51.6
Faeces 24 - 36	6.6	8.5
Faeces 36 - 48	2.9	3.5
Faeces 48 - 72	1.4	1.3
Subtotal faeces*	**89.6**	**84.5**
Total*	**106.4**	**102.4**

*Minor numerical deviations may occur due to rounding

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

The highest tissue concentration of radioactivity was found in the bone with a mean concentration of 49.8 µg equiv./g (male) and 44.9 µg equiv./g (female), followed by the gastrointestinal tract (GIT) plus contents with 13.3 µg equiv./g (male and 16.3 µg equiv./g (female). Lower mean concentrations between 1.1 and 6.6 µg equiv./g were found in kidneys, liver, heart, lungs, spleen, brain, gonads and salivary glands of both sexes. Mean concentrations of 4.8 and 5.9 µg equiv./g were found in the residual carcass (which also included the skeletal bone) of males and females respectively. All other concentrations were either similar to or lower than the

corresponding blood concentrations. The mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was < 0.6 % for both sexes. The amounts in the intestinal tract plus contents were about 0.2 % for both sexes. Please refer to table given below.

Table 6.1.1.4-8: Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat ([redacted]., 1996): Radioactivity in tissues after single oral dose of 1000 mg/kg bw at 72 h

	Males		Females	
Tissue	**% of dose**	**µg equiv./g**	**% of dose**	**µg equiv./g**
Blood	N/A	0.894	N/A	0.803
Bone	N/A	49.792	N/A	44.925
Brain	0.001	1.233	0.001	1.164
Fat	N/A	0.536	N/A	0.496
GIT plus contents	0.2	13.276	0.219	16.329
Heart	0.001	1.111	0.001	1.254
Kidneys	0.007	6.511	0.005	6.046
Liver	0.039	5.480	0.029	5.226
Lungs	0.002	2.870	0.002	3.535
Muscle	N/A	0.816	N/A	0.825
Ovary	-	-	<0.001	2.940
Plasma	N/A	<0.392	N/A	<0.396
Residual carcass	0.466	4.772	0.537	5.858
Salivary glands	<0.001	1.811	<0.001	2.089
Spleen	0.001	2.441	0.001	3.106
Testes	0.001	0.905	-	-
Total*	0.518	N/A	0.576	N/A

N/A not applicable
*Minor numerical deviations may occur due to rounding

C. RECOVERY OF RADIOACTIVITY

The total mean recovery, including excreta, tissues and residual carcass, was 107.1 % for male and 103.1 % for female rats.

III. CONCLUSIONS

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone having the highest tissue radioactivity.

Assessment and conclusion by applicant:

After single oral administration of 1000 mg/kg bw glyphosate to male and female rats, at least 17 % was absorbed (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 85 % of the dose are excreted *via* faeces, whereby again main portions are excreted within 24 h. No gender difference was noted.

Determination of concentrations in tissue 72 h after application revealed overall low levels each below 0.6 % of the dose (residual carcass) or 49.79 µg equiv./g (bone) for both genders. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Excretion essentially complete at 72 hours post dosing and most of it is excreted within the first 24 hours in faeces. Urinary excretion accounted for approximately 15-16% in the first 24 hours, which is much lower than the to the ~50% in animals dosed with 100 mg/kg bw of study CA 5.1.1/002
A similar distribution pattern in tissues was observed.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.5. Study 5

Data point	CA 5.1.1/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing
Report No	[redacted] P/4944
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	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 to be acceptable.

Executive summary

Eight male and eight female non-fasted rats received a single oral dose of 10 mg unlabelled glyphosate acid/kg bw daily for 14 days by gavage. 24 h after the 14th dose of unlabelled glyphosate acid, five rats/sex received a single oral dose of 10 mg/kg bw of [^{14}C]-phosphonomethyl-labelled glyphosate acid. After monitoring the excretion of radioactivity in urine and faeces for 72 h after dosing, the rats were sacrificed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.
The results showed that excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces, within 24 h. Over 72 h, males and females excreted means of 86.6 % and 90.7 % of the dose in faeces, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification: Glyphosate acid (N-phosphonomethyl glycine)
Description: White solid
Lot/Batch #: Y04707/045
Purity: 99.2 % w/w

Stability of test compound:	Not reported
2. Radiolabelled test material:	
Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>98 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in the vehicle for longer than a period of use during the study
3. Vehicle and/ or positive control:	Deionised water
4. Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Males and females
Weight at dosing (radiolabelled dose) :	225 - 328 g
Acclimation period:	At least 4 days prior to the study start and 24 h prior to dosing with the radiolabelled preparation
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Wiltham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation and treatment with unlabelled dose: Groups of the same sex, in stock rat cages, 24 h prior to dosing [^{14}C]-phosphonomethyl-labelled glyphosate acid transfer individually into metabolism cages After administration of [^{14}C]-phosphonomethyl-labelled glyphosate acid: Individually in stainless steel metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: At least 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: 1995-10-16 to 1996-03-26

Animal assignment and treatment

Eight male and eight female non-fasted rats received a single oral dose of 10 mg unlabelled glyphosate acid/kg bw daily for 14 days by gavage. 24 h after the 14th dose of unlabelled glyphosate acid, five rats/sex received a single oral dose of 10 mg/kg bw of [^{14}C]-phosphonomethyl-labelled glyphosate acid. 72 h after dosing, the rats were sacrificed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Collection of excreta

Urine was collected at 0 - 6, 6 - 12, 12 - 24, 24 - 36, 36 - 48 and 48 - 72 h h after dosing in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24, 24 - 36, 36 - 48 h and 48 - 72 h intervals.
Urine collections comprised rinsing of each cage at each time point with water together with a thorough washing at the end of the study using ethanol/water 1:1 (v/v).

Collection of blood and tissues

72 h after dosing, animals were exsanguinated by cardiac puncture under halothane anaesthesia. A blood sample was retained and divided into two portions. A portion of each blood sample was centrifuged to obtain plasma, which was analysed by liquid scintillation counting. Whole blood was analysed by sample oxidation.

The following tissues/organs were taken or sampled for radioactivity measurements: Bone (femur), brain, fat (abdominal), gastrointestinal tract and its contents, gonads, heart, kidneys, liver, lungs, muscle (femoral), spleen, salivary glands and residual carcass.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Measurement of radioactivity

Samples of urine, cage wash and plasma were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC. Bone (cut into pieces) and whole blood were analysed by sample oxidation followed by LSC. Liver, fat, residual carcass and gastrointestinal tract (GIT) plus contents were homogenised. Liver, fat and residual carcasses were than solubilised in Soluene-350® whereas GIT plus contents were oxidised. All other tissues were solubilised without prior homogenisation.

Liquid scintillation counting

Radioactivity was measured by liquid scintillation counting by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.

Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60: 30: 15:5 v/v/v/v).

Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mM aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample which was twice the liquid scintillation counter's background rate. For the purpose of calculating group mean results, individual values below the LOD are accepted as being equal to the limit of detection. Means which include one or more values which are below the LOD are reported as "<" the mean result and without a standard deviation. The limit of detection obtained for all tissues in this study was 0.004/µg equivalents glyphosate acid/g of tissue (µg equiv/g). This value is based upon a sample size of 200 mg of all determinations. Organs of less than this weight were analysed as a single sample and hence this figure represent a limiting value.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After repeated application of glyphosate acid excretion of the final phosphonomethyl-labelled glyphosate acid dose was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (average of 80.6 % for males and 85.8 % for females).

Excretion of radioactivity in the urine during this period accounted for means of 9.2 % and 9.1 % of the administered dose in male and female rats respectively. The total percentage of the administered radioactivity eliminated in excreta 72 h after dosing were means of 97.5 % for males and 101.7 % for females. Please refer to table given below.

Comparison of the cumulative excretion data showed that there were no marked differences in the rates of excretion of radioactivity in the urine or faeces for male and female rats.

Table 6.1.1.5-9: Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing ([redacted] 1996): Excretion of radioactivity (in mean % of applied dose) in urine and faeces in male and female rats treated with 10 mg/kg bw/day for 14 days

	Excretion of radioactivity [%]			
	Males			
Time after dosing	**Urine**		**Faeces**	
(hours)	**Mean[a]**	**SD**	**Mean**	**SD**
0 - 6	3.1	0.8	N/A	N/A
6 - 12	2.7	0.7	N/A	N/A
0 - 12	N/A	N/A	50.2	15.5
12 - 24	3.4	1.6	30.3	9.0
24 - 36	0.9	0.3	3.6	1.5
36 - 48	0.3	<0.1	1.3	0.7
48 - 72	0.2	<0.1	1.1	0.6
0 - 72	10.6	3.0	86.6	5.2
	Mean	**SD**	N/A	N/A
Cage wash at 72 h	0.2	<0.1	N/A	N/A
Total excreted*	97.5	2.7	N/A	N/A
	Females			
Time after dosing	**Urine**		**Faeces**	
(hours)	**Mean[b]**	**SD**	**Mean**	**SD**
0 - 6	3.3	0.6	N/A	N/A
6 - 12	2.5	0.3	N/A	N/A
0 - 12	N/A	N/A	44.7	34.1
12 - 24	3.2	0.6	41.0	31.1
24 - 36	0.9	0.3	2.7	1.1
36 - 48	0.4	0.2	1.2	0.2
48 - 72	0.3	0.1	1.1	0.7

0 - 72	10.7	1.1	90.7	4.2
	Mean	**SD**	N/A	N/A
Cage wash at 72 h*	0.2	<0.1	N/A	N/A
Total excreted*	101.7	4.0	N/A	N/A

N/A not applicable
[a] Mean of 4 animals
[b] Mean of 5 animals
* Minor numerical deviations may occur due to rounding

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

The highest tissue concentration of radioactivity was found in bone with a mean concentration of 0.36 µg equiv./g for males and 0.35 µg equiv./g for females, followed by the intestinal tract (GIT) plus contents with 0.11 and 0.12 µg equiv./g for males and females respectively. Lower mean concentrations between 0.02 and 0.06 µg equiv./g were found in kidneys, liver, lungs, salivary glands, and ovaries.

Mean concentrations of 0.05 µg equiv./g were found in the residual carcass of either sex which also includes the remaining skeletal bone.

All other concentrations were either similar to or lower than the corresponding blood concentrations.

The mean total percentage of administered radioactivity present in all of the tissues examined and the residual carcass was 0.5 % for males and 0.4 % for females. The amounts present in the intestinal tract plus contents were 0.1 % for males and females. Please refer to table given below.

Table 6.1.1.5-10: Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing [redacted] 1996): Tissue and carcass residues of radioactivity in male and female rats

	Residue of radioactivity			
Tissue	**Males**			
	% radioactivity of dose		**µg equivalents/g**	
	Mean [a]	**SD**	**Mean**	**SD**
Brain	0.001	<0.001	0.010	0.002
Testes	0.001	<0.001	0.007	0.001
Heart	<0.001	<0.001	0.011	0.002
Kidneys	0.005	0.002	0.061	0.015
Liver	0.031	0.009	0.055	0.014
Lungs	0.001	<0.001	0.026	0.004
Spleen	0.001	<0.001	0.022	0.003
Salivary glands	<0.001	<0.001	0.019	0.004
Bone (femur)	N/A	N/A	0.358	0.177
Fat (abdominal)	N/A	N/A	0.008	0.001
Muscle (femoral)	N/A	N/A	0.008	0.001
Blood	N/A	N/A	0.014	0.006
Plasma	N/A	N/A	<0.004	-
Residual carcass	0.423	0.090	0.050	0.011
Total	0.463	0.101	N/A	N/A
Intestinal tract plus contents	0.108	0.040	0.109	0.041
Tissue	**Females**			
	% radioactivity of dose		**µg equivalents/g**	
	Mean [b]	**SD**	**Mean**	**SD**
Brain	0.001	<0.001	0.010	0.002
Ovaries	<0.001	<0.001	0.026	0.006
Heart	<0.001	<0.001	0.012	0.004
Kidneys	0.004	0.001	0.049	0.011
Liver	0.021	0.005	0.045	0.010

Lungs	0.001	<0.001	0.029	0.006
Spleen	0.001	<0.001	0.025	0.006
Salivary glands	<0.001	<0.001	0.027	0.006
Bone (femur)	N/A	N/A	0.345	0.081
Fat (abdominal)	N/A	N/A	0.006	0.002
Muscle (femoral)	N/A	N/A	0.007	0.002
Blood	N/A	N/A	0.010	0.002
Plasma	N/A	N/A	<0.005	-
Residual carcass	0.382	0.067	0.046	0.008
Total	0.411	0.073	N/A	N/A
Intestinal tract plus contents	0.115	0.014	0.117	0.015

N/A not applicable
Residual carcass values include partial tissue percentages
[a] Mean of 4 animals
[b] Mean of 5 animals

C. RECOVERY OF RADIOACTIVITY

The total mean percentage recoveries, including excreta, tissues and residual carcass was 98.0 % for male rats and 102.2 % for females.

III. CONCLUSIONS

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Negligible traces of radioactivity (<0.6 %) were still present in the tissues and residual carcass at 72 h, with bone having the highest tissue radioactivity.

Assessment and conclusion by applicant:

After repeated oral administration of 10 mg/kg bw glyphosate to male and female rats for 14 consecutive days, about 11 % was absorbed (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 90 % of the dose was excreted *via* faeces, whereby again main portions were excreted within 24 h. No gender difference was noted.

Determination of concentrations in tissue 72 h after the last application revealed overall low levels each below 0.5 % of the dose (residual carcass) or 0.35 to 0.36 µg equiv./g (bone) for female and male rats.

Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance (see CA 5.1.1/003) showed no significant differences on either the routes or rates of elimination after oral dosing. In both studies the test substance was excreted rapidly and predominantly in the faeces by rats of both sex and low amounts of radioactivity were detected in all the tissue examined.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. There are no significant differences observed in animals following repeated exposure compared to single exposure to the compound. Therefore, the compound does not accumulate.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.6. Study 6

Data point	CA 5.1.1/006
Report author	[redacted]
Report year	1996

Report title	Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg)
Report No	[redacted]P/4943
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	Only 2 animals per dose per sex were tested which is below the 4 stated in the OECD 417 guideline.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** Unacceptable due to a too low number of animals.

Executive summary

Two male and two female rats were administered a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw. One male and one female was sacrificed 24 h after dosing and the other pair were sacrificed 48 h after dosing. Qualitative whole body autoradiogram was performed on all animals. In addition, radioactivity was measured in urine, faeces and exhaled air.

24 h after dosing, excreted means of the administered dose in the urine and faeces amounted to 22.3 % and 55.5 % in males and 11.9 % and 83.8 % in females, respectively, whereas less than 0.2 % was detected in exhaled air. 48 h after dosing, excretion of the administered dose in urine and faeces increased to 34.0 % and 60.5 % in males and 12.5 % and 91.2 % in females, respectively. The whole body autoradiograms showed no marked differences in the tissue distribution of radioactivity between male and female rats. The greatest intensity of radioactivity was present in the bone for both sexes, followed by the intestinal tract and the kidneys 24 h after dosing with lesser to negligible amounts being present after 48 h.

In conclusion, glyphosate acid was excreted rapidly and predominantly in the faeces. 24 and 48 h after dosing the greatest intensity of radiolabelling were found in the bone and intestinal tract and contents.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-phosphonomethyl glycine)
Description:	White solid
Lot/Batch #:	Y04707/045
Purity:	99.2 % w/w
Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	>96 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	Stable in the vehicle for longer than a period of use during the study

3. Vehicle and/ or positive control:	Deionised water

4. Test animals:

Species:	Rat
Strain:	Alpk:AP_fSD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Males and females
Weight at dosing:	215 - 271 g
Acclimation period:	At least 5 days in stock rat cages and 24 h prior to dosing in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After administration of [^{14}C]-phosphonomethyl-labelled glyphosate acid: Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: At least 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: 1995-10-25 to 1996-04-04

Animal assignment and treatment

Two male and two female non-fasted rats were administered with a single oral dose of 10 mg [^{14}C]-glyphosate acid/kg bw by gavage. 24 and 48 h after dosing, a heterosexual pair was sacrificed and a qualitative whole body autoradiogram was performed. In addition, radioactivity was measured in urine, faeces and exhaled air.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The (radiochemical) purity of the [^{14}C]-labelled test substance prior to and following formulation in the vehicle was determined by thin layer chromatography (TLC) high performance liquid chromatography (HPLC).

Collection of excreta

Urine was collected at 0 - 6, 6 - 12, 12 - 24 h from all animals and on 24 - 36 and 36 - 48 h after dosing of two animals in receivers cooled with solid CO_2. Faeces were collected at 0 - 12, 12 - 24 h from all animals and on 24 - 36, 36 - 48 h intervals h after dosing of two animals.
Urine collections comprised rinsing of each cage at each time point with water together with a thorough washing at the end of the study using ethanol/water 1:1 (v/v).

Collection of exhaled air

The exhaled air from one heterosexual pair was passed through sodium hydroxide to trap any radioactivity expired as [^{14}C]-carbon dioxide. Subsamples of the contents of each trap were removed for radiochemical analysis at 6, 12, and 24 h after dosing.

Whole body autoradiography

Immediately after scheduled sacrifice, each carcass was frozen rapidly and embedded in blocks of 2 % (w/v) aqueous carboxymethylcellulose. Longitudinal sagittal sections, 30 µm thick, were taken, mounted on adhesive tape and freeze-dried for approximately 48 h. Autoradiograms were prepared by contact with autoradiographic film and exposed for periods of 2, 4 or 6 weeks before fixing and washing.

Measurement of radioactivity

Samples of urine, cage wash and exhaled air were taken, without further processing, for liquid scintillation counting (LSC). Faecal samples were ground with an approximately equal weight of anhydrous magnesium sulphate until homogeneous. Samples were analysed by sample oxidation followed by LSC.

Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60:30:15:5 v/v/v/v).
Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser. Unlabelled glyphosate acid was visualised by spraying the TLC plate with a 0.2 % ethanoic ninhydrin solution.

High performance liquid chromatography (HPLC)

To facilitate analysis a mixture of the unlabelled and radiolabelled test substance was derivatised. Sample analysis was performed by a Hichrom S5NH column (250 x 4.6 mm) which was eluted with acetonitrile buffered with 25 mM aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min. Radioactivity was detected using an on-line flow detector (liquid cell) and with UV absorption at 230 nm.

Data evaluation

Data were processed using the Debra (Version 4.1) computerised acquisition and processing system. The limit of detection (LOD) of radioactivity measurement during this study was taken as 50 dpm per sample, which was twice the liquid scintillation counter's background rate. The LOD for each carbon dioxide trap in this study was 0.01 % of the administered dose.

II. RESULTS AND DISCUSSION

A. EXCRETION OF RADIOACTIVITY

Excretion was rapid for both sexes with most of the radioactivity being eliminated in the faeces during the first 24 h after dosing (average of 55.5 % for males and 83.8 % for females). Excretion of radioactivity in the urine during this period accounted for means of 22.3 % and 11.9 % of the administered dose in male and female rats respectively. Less than 0.2 % of the applied dose was detected in exhaled air.

After 48 h, 60.5 % and 91.2 % of the applied dose were detected in faeces of the male and the female rat, respectively. 34.0 % and 12.5 % were detected in urinary samples of the male and the female rat, respectively. Urinary radioactivity after 48 h accounted for 34 % of the applied dose in one male rat while the urinary radioactivity in the other three animals was below 19 % each.

Individual results of the excreted radioactivity in urine, faeces and exhaled air expressed as percentages of the administered radioactivity, together with the results for cage washings, are listed in the table given below.

Table 6.1.1.6-11: Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg) [redacted] 1996): Excretion of radioactivity in urine, faeces, cage wash and expired air by male and female rats at 10 mg/kg bw

	Time After Dosing (hours)	Males		Females	
		Rat 1	Rat 2	Rat 3	Rat 4
		(% of applied dose)		(% of applied dose)	
Urine	**0 - 6**	5.00	8.21	4.06	3.67
	6 - 12	5.30	8.67	4.32	4.12
	12 - 24	7.57	9.92	4.39	3.23
	24 - 36	N/A	4.30	N/A	0.93
	36 - 48	N/A	2.90	N/A	0.54
	Total	17.86	34.00	12.77	12.48
	Terminal Cage wash	1.12	0.98	1.43	0.41
Total excreted*		18.98	34.98	14.10	12.89
Faeces	**0 - 12**	30.62	0.21	54.27	32.63
	12 - 24	28.69	51.48	26.03	54.76
	24 - 36	N/A	1.65	N/A	2.71
	36 - 48	N/A	7.12	N/A	1.13
	Total	59.32	60.46	80.30	91.23
Exhaled air	**0 - 6**	0.07	N/A	0.08	N/A
	6 - 12	0.02	N/A	0.04	N/A
	12 - 24	0.02	N/A	0.03	N/A
	Total	0.11	N/A	0.14	N/A
Total*		78.40	95.44	94.64	104.12

N/A not applicable
*Minor numerical deviations may occur due to rounding

B. WHOLE BODY AUTORADIOGRAPHY

The whole body autoradiograms showed no marked differences in the distribution of radioactivity between male and female rats. The greatest intensity of radioactivity was present in the bone for both sexes, as well as the intestinal tract and the kidneys 24 h after dosing with lesser to negligible amounts being present after 48 h.

III. CONCLUSIONS

Oral doses of glyphosate acid were excreted rapidly and predominantly in the faeces. The remaining radioactivity was excreted with the urine. Elimination was essentially complete within 72 h. Only low amounts (<0.2 % of the applied dose) were detected in exhaled air indicating only a low metabolism to $^{14}CO_2$ 24 h after dosing the greatest intensity of radioactivity was observed in the bone, the intestinal tract plus contents and kidneys. The intensity decreased within 48 h.

Assessment and conclusion by applicant:

After single oral administration of 10 mg/kg bw glyphosate acid to male and female rats, about 12 % and 22 % were absorbed by female and male rats respectively (based on the amount excreted *via* urine including cage wash). Urinary excretion is mainly completed within 24 h. About 85 % and 60 % of the dose are excreted *via* faeces within 48 h by females and male respectively, whereby again main portions are excreted within 24 h. Urinary excretion of radioactive labelled material in males is higher compared to females. However this is due to the high urinary excretion of one male rat (35 %). Gender specific differences in absorption were not observed in any of the other available studies and given the limited number of animals per sex in this study this is considered incidental.

The highest intensity of radioactivity in the whole body autoradiography was observed in the bone, the intestinal tract plus contents and kidneys. This confirms the tissue distribution observed in other studies.
The decrease in radioactivity after 48 h indicates a fast elimination from the body and thus do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:
Due to the limitations, the study is considered unacceptable. However, the results confirm the outcome of the previously discussed studies.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.7. Study 7

Data point	CA 5.1.1/007
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Biotransformation in the rat
Report No	[redacted]P/5058
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985), OECD 417 (1984), US-EPA FIFRA 85-1, EEC B.36 (1987)
Deviations from current test guideline	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 to be acceptable.

Executive summary

[^{14}C]-labelled glyphosate was administered by gavage as a single dose of 1000 mg [^{14}C]-glyphosate acid/kg bw to two male and two female bile duct cannulated rats. Bile was collected at various time intervals for 48 h. Additionally urine and faeces were collected at various intervals and the proportion and ratio of metabolites were investigated.

To further investigate the biotransformation of glyphosate acid, samples of urine and faeces of former studies (CA 5.1.1/003, CA 5.1.1/004, CA 5.1.1/005) were pooled and analysed by chromatography and NMR for metabolites.

The results showed that following a single oral dose of [^{14}C]-glyphosate acid the excretion of radioactivity in bile is negligible, thus indicating that radioactivity recovered in faeces is most likely unabsorbed material. The radioactivity present in urine and faeces from rats given [^{14}C]-glyphosate acid at low or high dose levels or after repeated dosing was characterised as being predominantly glyphosate acid. Trace amounts of aminomethylphosphonic acid (AMPA) were detected in urine samples.

In conclusion, following an oral dose of glyphosate acid to male and female rats approximately 10 - 20 % of the dose was absorbed. The unabsorbed glyphosate acid was excreted unchanged in faeces. The absorbed dose was excreted in urine as glyphosate acid and trace amounts of AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate acid (N-(phosphonomethyl) glycine)
Description:	White solid
Lot/Batch #:	Y04707/048
Purity:	99.5 % w/w
Stability of test compound:	Not reported, but used within the stated expiry date.

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate acid
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Y04707/047
Radiochemical purity:	97.8 %
Specific activity :	1.580 GBq/mMol
Stability of test compound:	The test substance was shown to be stable in vehicle for longer than the period of use during this study

3. Vehicle and/ or positive control:

Deionised water

4. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

4. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	[redacted]
Age:	Not reported, but at least 6 weeks according to body weight
Sex:	Males and females
Weight at dosing:	260 - 305 g
Acclimation period:	At least 4 days in stock rat cages and 24 h prior to surgery in metabolism cages
Diet/Food:	Pelleted PCD rat diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of the same sex, in stock rat cages, 24 h prior to dosing transfer individually into metabolism cages After dosing: Housed individually in glass metabolism cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 - 70 % Air changes: At least 12/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: 1995-11-26 to 1996 May

Animal assignment and treatment

Four non-fasted rats (2 males, 2 females) received single oral doses of 1000 mg [^{14}C]-glyphosate acid/kg bw by gavage after bile duct cannulation. 48 hours after dosing all animals were sacrificed.

Bile duct cannulation

The abdominal cavity was opened after anaesthesia and the bile duct exposed. A fine plastic cannula was inserted into the bile duct and externalised by passing through the abdominal wall and under the skin to an exit point at the back of the neck. The incisions in the abdominal and body walls were saturated and the exposed cannula was protected within a flexible metal sheath anchored to the skin at the back of the neck. Following surgery each animal was returned to its cage and allowed to recover overnight prior dosing.

Collection of excreta and bile

Urine was collected 0 - 6, 6 - 12, 12 - 24, 24 - 36 and 36 - 48 intervals after dosing. Faeces was collected at 0 - 12, 12 - 24, 24 - 36 and 36 - 48 h intervals after dosing.
Bile was collected at 0 - 2, 2 - 4, 4 - 6, 6 - 8, 8 - 12, 12 - 24, 24 - 36, and 36 - 48 h intervals after dosing.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation was determined by liquid scintillation counting. The radiochemical purity of the [^{14}C]-labelled test substance was determined by the high performance liquid chromatography (HPLC) following formulation in the dosing.

Quantification of metabolites

Urine and faecal samples obtained from the excretion and tissue distribution studies described in Sections CA 5.1.1/003 – 005 over 72 hours were used for the quantification of metabolites.
Urine samples from each study were combined by taking a fixed percentage by weight to give separate male and female pools for each of the sample collections intervals. Subsamples of these pools were further combined to give pools representing the entire sample collection period. Each pool was analysed by TLC and HPLC. A representative urine sample was analysed by ^{1}H-NMR.
Faecal samples were combined in the same way as described above for urine samples. Subsamples of pooled faecal samples were mixed with distilled water and sonicated for several hours, the samples were filtered through filter paper and the solid material was re-extracted a second time with distilled water and a third time with 10 % aqueous HCl. Extract volumes were measured and aliquots taken for scintillation counting to allow the calculation of extraction efficiencies.

Measurement of radioactivity

Faecal samples were extracted with water twice and the pooled supernatants were analysed by sample oxidation followed by liquid scintillation counting (LSC) whereas samples of urine and bile were analysed without intermediate processing by LSC.

Liquid scintillation counting

Radioactivity was measured by liquid scintillation analysis by means of Packard Tricarb instruments. The results obtained were corrected for background activity and counting efficacy using [^{133}Ba] as the external source. Disintegrations per minute (dpm) values were calculated using the appropriate quench curve data entered into instrument's computer.
Where sample oxidation had to be performed, samples were oxidised in a Packard Tricarb sample oxidiser.

Thin layer chromatography (TLC)

TLC was conducted using a normal phase silica-gel ($60F_{254}$) with the following solvent system: methanol: water: 28 % ammonium hydroxide: 10 % trichloroacetic acid (60:30:15:15 v/v/v/v).
Radioactivity on the TLC plate was measured using a Berthold Tracemaster linear analyser or a Bioscan System 200 imaging scanner. Glyphosate acid and AMPA standards were located by spraying the plates with a solution of 300 mg ninhydrin in 100 mL of butanol and 3 mL of glacial acetic acid.

High performance liquid chromatography (HPLC)

Two different HPLC methods were employed:
HPLC method 1 was used for the analysis of dosing solutions. Prior to analysis samples were derivatised. A Hichrom S5NH column (250 x 4.6 mm) was eluted with acetonitrile buffered with 25 mM aqueous potassium dihydrogen phosphate (60:40 v/v) at a flow rate of 1.5 mL/min.
HPLC method 2 was used for the analysis of urine and faecal extracts and for the quantification of glyphosate acid and AMPA. Prior to analysis samples were filtered. A Biorad's HLRC acid analysis column (250 x 4.6 mm) was eluted with 5 mM aqueous potassium dihydrogen phosphate with 4 % methanol at a flow rat of 0.5 mL/min.
Radioactivity was detected in both methods by liquid cell.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR)

Proton and phosphorus NMR spectra were acquired using a Brucker 400MHz instrument. Samples of glyphosate acid and AMPA were dissolved in D_2O and analysed by both phosphorus and proton NMR. Control urine and urine from a bile duct cannulated rat administered an oral dose of glyphosate acid were analysed by phosphorus NMR. The urine sample from the rat that had been administered glyphosate was subsequently fortified with AMPA then glyphosate acid and reanalysed by phosphorus NMR.

Data evaluation

Dosing and excretion of radioactivity data were processed using the Debra computerised acquisition and processing system. Metabolites were quantified using the Flo_One integration software for HPLC.

II. RESULTS AND DISCUSSION

A. EXCRETION OF RADIOACTIVITY

48 h after dosing, mean recovery of the administered dose in the urine amounted to 20.8 % and 16.3 % in males and females, respectively. In faeces 39.1 % and 30.5 % of the applied dose were detected in males and females, respectively. The total excreted radioactivity after 48 h accounted for 62.5 % and 52.0 % of the applied dose in males and females, respectively.

Biliary excretion of radioactivity was negligible (about 0.06 %). Please refer to table given below.

Table 6.1.1.7-12: Glyphosate acid: Biotransformation in the rat [redacted] 1996): Excretion of radioactivity in urine, faeces and bile by male and female bile duct cannuled rats given a single oral dose of 1000 mg [^{14}C]-glyphosate acid/kg bw (mean of two rats expressed as % of applied dose)

Time after dosing (hours)	Males % of applied dose			Females % of applied dose		
	Urine	Faeces	Bile	Urine	Faeces	Bile
0 - 2	N/A	N/A	0.004	N/A	N/A	0.002
2 - 4	N/A	N/A	0.004	N/A	N/A	0.011
4 - 6	N/A	N/A	0.002	N/A	N/A	0.011
0 - 6	2.137	N/A	N/A	8.718	N/A	N/A
6 - 8	N/A	N/A	0.005	N/A	N/A	0.005
0 - 12	N/A	3.776	N/A	N/A	1.392	N/A
6 - 12	6.765	N/A	N/A	2.495	N/A	N/A
8 - 12	N/A	N/A	0.008	N/A	N/A	0.007
12 - 24	5.432	12.333	0.016	3.631	12.115	0.010
24 - 36	3.468	18.079	0.009	1.004	8.712	0.008
36 - 48	3.013	4.946	0.007	0.427	8.325	0.007
0 - 48	**20.185**	**39.134**	**0.055**	**16.275**	**30.544**	**0.062**
Cage wash at 48 hours	**2.534**			**5.097**		
Total urinary excretion	**22.719**	N/A	N/A	**21.372**	N/A	N/A
Total excretion	**62.538**			**51.978**		

Values are expressed as percentages of administered dose and are then mean of two rats
N/A: Not applicable

B. CHARACTERISATION OF RADIOACTIVITY

Due to the low level of radioactivity in bile samples the radioactivity in bile was not further characterised.

From pooled faeces samples of the low dose application (10 mg/kg bw) 84.5 and 62.3 % of the radioactivity for males and female was extracted. 88.5 and 75.6 % of the radioactivity was extracted from the pooled faeces of the study investigating excretion of ^{14}[C]-glyphosate acid at the high dose (1000 mg/ kg bw) from males and females. From faeces of the repeated application of ^{14}[C]-glyphosate acid 61.0 and 79.5 % of the radioactivity was extracted.

Analyses by chromatography and phosphorus NMR of urine pools from those former studies (see CA 5.1.1/003; CA 5.1.1/004; CA 5.1.1/005) covering the 0 - 72 h period demonstrated a single peak identified as glyphosate acid by phosphorous NMR and a fortification experiment. Chromatograms of urine collected at earlier intervals demonstrated a second peak that occurred in measurable quantities. The peak was identified as aminomethylphosphonic acid (AMPA) by co-chromatography.

The percentages of dose accounted for glyphosate acid and AMPA following a low, high or repeated dose of glyphosate acid are given in the table below. For glyphosate acid and AMPA the values range from 63.3 - 95.3 % and 0.07 - 0.66 %, respectively.

Table 6.1.1.7-13: Glyphosate acid: Biotransformation in the rat ([redacted] 1996): Percentage of administered radioactivity identified as glyphosate acid and AMPA

		Low dose study 10 mg/kg bw		High dose study 1000 mg/kg bw		Repeated dose study 10 mg/kg bw	
		Male	Female	Male	Female	Male	Female
		(% of applied radioactivity)					
Urine	**Glyphosate acid**	12.71	10.51	16.00	16.73	10.46	10.47
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08
Faeces	**Glyphosate acid**	74.80	55.22	79.25	63.88	52.86	72.09
Total	**Glyphosate acid**	87.52	65.73	95.25	80.61	63.33	82.57
	AMPA	0.19	0.11	0.63	0.66	0.07	0.08

Assessment and conclusion by applicant:

Following an oral dose of glyphosate acid to rats approximately 10 - 20 % of the dose was absorbed (see CA 5.1.1/003, CA 5.1.1/004, CA 5.1.1/005). The absorbed dose was excreted in urine as glyphosate and trace amounts of aminomethylphosphonic acid (AMPA). No significant quantities of glyphosate acid were eliminated in bile, which confirms that faecal radioactivity represents unabsorbed dose. The radioactivity excreted via faeces was confirmed to be glyphosate acid.

Assessment and conclusion by RMS:

Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Taking into account the negligible biliary excretion, oral absorption is likely represented by renal elimination. There are no notable differences in male and female animals.
Glyphosate is mainly excreted without metabolization. A small part of the absorbed compound is excreted as AMPA.
This conclusion is in line with the previous assessment (RAR, 2015).

B.6.1.1.8. Study 8

Data point	CA 5.1.1/008; CA 5.1.1/009
Report author	Part 1: [redacted] (*in vivo* part) Part 2: [redacted] (Metabolite analysis)
Report year	1995
Report title	Part 1: Metabolism Study of ^{14}C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats Part 2: Glyphosate - ADME-Study in Rats
Report No	Part 1: 9202/95 Part 2: [redacted] 038/94
Document No	Not reported
Guidelines followed in study	None reported
Deviations from current test guideline	Yes, reporting and methodological deficiencies
Previous evaluation	Yes, supplementary in RAR (2015)

GLP/Officially recognised testing facilities	Part 1: Yes Part 2: Yes, self-certification (Appendices in report missing)
Acceptability/Reliability	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. However, the study is considered supplemental due to reporting and methodological limitations. The radioactivity was only investigated in the organs, tissues and carcass in one group receiving a low intravenous dose. Additionally, radioactivity in the bone was not determined. Also, the investigation of possible metabolites was only performed in the urine and not in faeces.

Executive summary

The objective of this study was the investigation of the absorption, distribution, metabolism and excretion of glyphosate in rats upon low and high dose oral application and low dose intravenous application. The study was performed according to the ADME scheme (Adsorption, Distribution, Metabolism, Excretion).

Groups of 4 male and 4 female Sprague-Dawley rats were used for Groups I to III. In Group I [^{14}C]-glyphosate was administered by intravenous bolus injection at ~0.2 mg/kg bw. In Groups II and III, [^{14}C]-glyphosate was administered by oral gavage at ~0.2 mg/kg and ~200 mg/kg bw, respectively.

The mean recovery of administered radioactivity in excreta (urine and faeces) exceeded 90 % in all investigations. Seven days after single intravenous dosing, elimination in urine accounted for ca 89.3 % of the dose compared with 6.3 % in faeces. After oral dosing 83.1 % of the administered [^{14}C]-glyphosate were detected in faeces and 10.9 - 15.1 % in urine. Renal elimination was more protracted following oral administration of [^{14}C]-glyphosate continuing for at least 24 h post-dose. A mean of 89 % of the dose was excreted with the urine and faeces within 24 h following administration.

The proportion of radioactive dose absorbed from the gut, estimated by comparison of renal elimination following single intravenous and oral administration at ~0.2 mg/kg bw, was 12 %.

Total radioactivity in organs 7 days after single intravenous administration of ~0.2 mg/kg bw (Group IV, 8 female rats) was low (<0.3 % of dose), however only a limited number of tissues were analysed.

No radiolabelled metabolites of [^{14}C]-glyphosate were detected in urine of the high dose animals collected within 24 h. The identity of the radioactive component as glyphosate was confirmed by HPLC and thin layer chromatography.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	White powder
Lot/Batch #:	Part 1: UN-NO: 1759 Part 2: 32140
Source:	Part 1: Not reported Part 2: [redacted]
Purity:	98 %
Stability of test compound:	Stable for duration of the study

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	Not reported
Specific activity:	11.9 and 11.7 MBq/mg (2.04 and 2.00 GBq/mmol)
Stability of test compound:	Stable for duration of the study
3. Reference substances:	
Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	118F3838
Purity:	Not reported
Stability of test compound:	Stable for duration of the study
4. Vehicle and/ or positive control:	Groups 1, 2, 4: 250 µL water Group 3: 2.75 mL aqueous tylose + 250 µL glyphosate in water
5. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR)
Source:	[redacted]
Age:	5 - 7 weeks at start of experiment
Number/Sex:	12 males and 20 females
Weight at dosing:	190 - 209 g
Acclimation period:	7 days
Diet/Food:	Altromin 1324; rats were fasted for approximately 16 h prior to administration
Water:	Tap water, *ad libitum*
Housing:	During acclimation: single housing in Makrolon type III cages After administration in metabolism cages
Environmental conditions:	Temperature: 22 ± 3ºC (at room temperature) Humidity: 60 ± 20 % Air changes: Not reported 12-hour light/12-hour dark cycle

B. STUDY DESIGN

In life dates: 1995-06-07 to 1995-06-13

Animal assignment and treatment

The test substance glyphosate was administered to four groups of animals. Groups I to III consisted of four males and four females, Group IV comprised eight females. The test substance was administered according to the following scheme:

Group	Route of administration	Amount of test substance (per animal)	Radioactive dose** (per animal)	Dose** (mg/kg bw)	Samples
I	i.v.	0.04 mg [^{14}C]-glyphosate	501 kBq	~0.2	Urine

II	Oral	0.05 mg [^{14}C]-glyphosate	702 kBq	~0.2	and faeces
III	Oral	0.04 mg [^{14}C]-glyphosate plus 40.0 mg unlabelled glyphosate*	501 kBq	~200	
IV	i.v.	0.04 mg [^{14}C]-glyphosate	501 kBq	~0.2	Blood and organs

* The unlabelled glyphosate was mixed with the labelled compound just before application.
** Doses as reported in the study report by Blech, 1995 (Part 2)

Preparation of the solution of the test substance

18.5 MBq (1.55 mg) of the test substance (specification of the supplier) dissolved in water were made up to volume with water in a 10 mL volumetric flask. The specific radioactivity of the solution was determined to be 2.005 MBq/mL. The unlabelled glyphosate was mixed with the labelled compound just before application.

Sampling

From the animals of Groups I to III, urine and faeces were collected over 7 days in 24 h fractions. The urine volumes per collection fraction and animal were determined. At the end of each collection period the cages were rinsed with 25 mL aqua *ad injectabilia*. The rinsing water volume per collection fraction and animal was determined.
Animals of Group IV were used for blood and tissue sampling. Two females were sacrificed per sampling time point at 1, 4, 24, and 168 h after administration for sampling of blood and the following organs: brain, spinal cord, fat, kidneys, liver, heart, ovaries. Carcasses were also sent to the analytical laboratory.

Measurement of radioactivity

Urine and rinsing water of rat cages
Aliquots of 0.5 - 1 mL of each sample (urine and rinsing water) were mixed with 4 mL of the scintillation cocktail Pico-Fluor 40. The scintillation was measured for 5 - 10 minutes. A quench curve prepared by internal standard measurement was used to calculate the content of radioactivity of the samples in disintegration per minute (dpm).

Faeces
The faeces samples were homogenised. Aliquots of approx. 0.5 g of each faeces sample were incinerated in a Sample Oxidizer Model 307 (Canberra Packard). The instrument combusts the sample material in a continuous flow of oxygen to trap the CO_2 of the incineration gas using a specially designed reaction column filled with 10 mL of 3-methoxypropylamine as carbon dioxide absorbent. 10 mL of a liquid scintillator for ^{14}C-counting is added automatically. The scintillation of the samples was measured for 5 - 10 minutes.

Organs
Following organs were collected from dose Group IV at 1, 4, 24 and 168 h after treatment, weighed and dissolved using tissue solubiliser: Heart, kidneys, ovaries, fat, brain, liver, and spinal cord. Most of the tissues were completely dissolved after 48 h, only the kidneys required 120 h. The solutions were weighed again and aliquots of approx. 1 mL were mixed with 4 mL of the scintillation cocktail Pico-Fluor 40 to measure their scintillation with the beta counter for 5 - 10 minutes.

Blood
The blood samples were homogenised, weighed and aliquots of approx. 1 g were dried and incinerated as described for the faeces samples.

Determination of absorption

Absorption was determined by comparing the renal excretion after intravenous and oral application.

Metabolite pattern of urine samples

The urine of Group III collected 24 h after application was analysed for metabolites and for un-metabolised test substance. The urine samples were analysed by HPLC with radioactivity monitoring using a Sherisorb 5 SAX column with 5 mM phosphate buffer (pH 1.9) /methanol (96:4) as eluent. In addition, the radioactive HPLC-fraction was collected and converted with 9-fluorenylmethyl chloroformate (FMC). The reaction mixture was separated by thin-layer chromatography. And radioactivity was monitored.

II. RESULTS AND DISCUSSION

A. ABSORPTION

Table 6.1.1.8-14: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats ([redacted], 1995): Cumulative total excretion of radioactivity (group means) after 7 days as % of dose

Sample	Group I i.v. dose ~0.2 mg/kg bw*	Group II Oral dose ~0.2 mg/kg bw*	Group III Oral dose ~200 mg/kg bw*
Urine incl. rinsing	89.3 ±3.4	10.9 ±2.3	15.1 ±4.0
Faeces	6.3 ±3.5	83.1 ±4.2	83.1 ±9.8
Total recovery	**95.6** ±2.1	**94.0** ±3.0	**98.2** ±11.3

* Doses as reported in the study report by [redacted] 1995 (Part 2)

The renal excretion of total radioactivity based on the administered parent compound plus metabolite(s) was measured both after oral and intravenous application to rats (Groups I to III). Comparing the urinary excretion after intravenous (Group I) and oral applications (Group II), an absolute bioavailability of about 12 % was calculated. In order to investigate the impact of the dose level on the absorption process, an approximately 800 times higher dose was given orally (Group III). The mean of the excretion rate in urine for Group III was determined to be 15 % of the dose. Comparing Group II and Group III, the absorption rate observed for the high orally administered Group III increased insignificantly about 4 % of the given dose, although the dose was 800 times higher.

B. ELIMINATION

Table 6.1.1.8-15: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats [redacted] 1995): Means of renally excreted radioactivity (urine and rinsing water) as % of dose

Group	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
I (i.v.)	M	85.2	2.43	1.05	0.55	0.38	0.23	0.2	90.03
	F	84.88	1.78	0.8	0.5	0.33	0.23	0.18	88.68
	M+F	**85.04**	**2.10**	**0.93**	**0.53**	**0.35**	**0.23**	**0.19**	**89.37**
II (oral)	M	11.18	0.78	0.15	0.1	0.05	0	0	12.25
	F	8.7	0.625	0.1	0.1	0	0	0	9.53
	M+F	**9.94**	**0.70**	**0.13**	**0.10**	**0.05**	**0.00**	**0.00**	**10.92**
III (oral)	M	15.5	1.03	0.25	0.1	0.1	0.08	0.03	17.08
	F	11.73	0.98	0.18	0.10	0.08	0.03	0.00	13.08
	M+F	**13.61**	**1.00**	**0.21**	**0.10**	**0.09**	**0.05**	**0.01**	**15.07**

M = males, F = females

Independently of route or dose level, most of the renally excreted radioactivity was found in urine during the first 24 h period (Group I: >93 %, Group II: >88 %, Group III: >83 %). The findings of excretion via faeces can be seen in the table below.

Table 6.1.1.8-16: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats [redacted] 1995): Means of radioactivity excreted via faeces as % of dose

Group	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
I (i.v.)	M	4.36	0.74	0.18	0.10	0.05	0.06	0.07	5.55
	F	6.08	0.53	0.19	0.13	0.09	0.08	0.05	7.14
	M+F	**5.22**	**0.64**	**0.18**	**0.12**	**0.07**	**0.07**	**0.06**	**6.36**
II (oral)	M	79.39	3.21	0.20	0.03	0.01	0.01	0.01	82.85
	F	79.56	3.40	0.17	0.10	0.02	0.01	0.01	83.27
	M+F	**79.47**	**3.30**	**0.18**	**0.06**	**0.02**	**0.01**	**0.01**	**83.05**
III (oral)	M	77.98	3.34	0.34	0.08	0.04	0.02	0.01	81.81
	F	81.93	2.20	0.14	0.04	0.03	0.02	0.01	84.36
	M+F	**79.95**	**2.77**	**0.24**	**0.06**	**0.03**	**0.02**	**0.01**	**83.08**

M = males, F = females

The total radioactivity recovered was 96, 94 and 98 % of the applied dose for Groups I, II and III, respectively.

C. DISTRIBUTION

The dispersal of radioactivity into blood and organs was measured at different times after administration. The mean measured radioactivity of organs collected at 1, 4, 24 and 168 h after dosing is provided in the table below.

Table 6.1.1.8-17: Metabolism Study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley Rats ([redacted] 1995): Summary of the organ distribution of radioactivity (means of the groups, spec. radioactivity as % of dose per gram tissue [%/g])

Group	Animal No	Time after administration	Brain	Spinal cord	Fat	Kidneys	Liver	Heart	Ovaries	Blood	Total
IV (i.v.)	25-26	1 h	0.020	0.025	0.027	5.351	0.132	0.063	0.077	0.119	**5.814**
	27-28	4 h	0.006	0.009	0.005	0.851	0.112	0.012	0.026	0.021	**1.042**
	29-30	24 h	0.005	0.009	0.002	0.025	0.058	0.004	0.006	0.008	**0.117**
	31-32	7 d	0.005	0.006	0.000	0.004	0.004	0.001	0.002	0.004	**0.026**

1 h after application, most organs showed a rather low radioactivity: 0.02 % to 0.13 % of the dose per gram of brain, spinal cord, fat, liver, heart, ovaries, and blood. The concentrations of radioactivity in kidneys was remarkably higher. It was determined to be 5.4 % of the dose/gram. 4 h after application, the radioactivity of the organs has declined, compared to the previous sampling. Radioactivity in the kidney was approx. 0.9 % of the dose per gram 4 h after intravenous application. The remaining organs were containing <0.1 % of the dose/gram of tissue. After 24 h, only liver and kidney exhibited a noticeable amount of approx. 0.06 % and 0.03 % radioactivity of the dose. After 7 days, none of the tissue samples showed a significant radioactivity (below 0.01 % of the dose/gram).

D. METABOLISM

The metabolism of glyphosate was studied by examination of the urine fractions of Group III animals. One aspect was the partition of the total urine radioactivity into the parent compound and its metabolites. When one or more metabolites were observed, an attempt to identify the main metabolite(s) was made. No metabolites were detected in the urine samples of Group III (0 - 24 h) analysed by HPLC. [^{14}C]-Glyphosate was identified by co-chromatography of the labelled reference substance under the same HPLC conditions. Upon conversion of the HPLC-fraction with 9-fluorenylmethyl chloroformate (FMC) and separation by thin-layer chromatography the FMC-derivative of [^{14}C]-glyphosate accounted for 94 % of the total counts.

III. CONCLUSIONS

Absorption, metabolism and elimination were independent of the oral dose in the range of 0.2 to 200 mg/kg bw. The radioactivity of the given test substance was eliminated renally and via faeces with a ratio of 15:1 after intravenous application. In the case of oral administration, the ratio was determined to be 1:8 after giving a low dose of approximately 0.2 mg/kg bw and 1:6 after a high dose of approximately 200 mg/kg bw. The systemic availability was found to be 11 % and 15 %, respectively. The absolute bioavailability was found to be 12 % comparing the low oral and intravenous application. The distribution of the radioactivity to several organs

showed high concentrations in the kidneys as excretory organs in the first 24 h after application. In urine no other radioactive compounds than the test substance could be detected. Unchanged glyphosate is very rapidly excreted.

Assessment and conclusion by applicant:

An ADME (absorption, distribution, metabolism and excretion) study in Sprague-Dawley rats was conducted. A single intravenous or oral dose of [^{14}C]-glyphosate at approximately 0.2 mg/kg bw or a single oral dose of 200 mg/kg bw were administered to Sprague-Dawley rats.

At 7 days after single i.v. dose at ~0.2 mg/kg bw the mean urinary excretion of glyphosate was 90.0 % in males and 88.7 % of the applied dose in females; in faeces, excretion was 5.6 % and 7.1 % of the applied dose. After single oral dose at ~0.2 mg/kg bw the mean urinary excretion of glyphosate over the collection period of 7 days was 12.3 % in males and 9.5 % of the applied dose in females; in faeces, excretion was 82.9 % (males) and 83.3 % (females) of the applied dose.
After single oral dose at ~200 mg/kg bw the mean urinary excretion of unchanged glyphosate was 17.1 % in males and 13.1 % of the applied dose in females; in faeces excretion was 81.8 % and 84.4 % of the applied dose. No metabolites were identified in urine collected within 24 h after application

The selection of tissues sampled for evaluation after single i.v. dose at ~0.2 mg/kg bw was limited. Highest tissue levels were detected after 1 h in the kidneys. 4 h after application, radioactivity in the organs had already started to decline. After 24 h, only the kidneys and the liver exhibited a noticeable amount of approximately 0.06 % or 0.03 % of the total radioactivity administered. After 7 days, only trace amounts of radioactive residues were detected, with highest concentration of 0.006 % of the dose detected in spinal cord.

Thus, elimination from the body was rapid after intravenous application and did not indicate a potential for bioaccumulation of glyphosate.

Urinary radioactivity collected within 24 h of the high dose animals was identified as glyphosate by HPLC and TLC. No metabolites were detected in urine.

In conclusion, absorption of glyphosate after oral application is rapid and independent of dose level. Absorption is low (approximately 12 - 15 % of the applied dose was absorbed). The majority of glyphosate is excreted unchanged within the first 24 h after application.

Dosing was done per animal, but individual body weights were not provided. In Part 1 (the report by [redacted] body weights were indicated to range between 190 - 209 mg and the low dose was assigned as ~0.2 mg/kg bw. In Part 2 (the report by [redacted]) the low dose was assigned as ~0.3 mg/kg bw, however this laboratory received only samples for analyses and may not have received information on individual body weights.

The report does not contain any information on the limits of detection or limits of quantitation. Metabolites were only assessed in urine samples from the oral high dose group. The low dose of only ~0.2 mg/kg bw used in this study was rather low, but the use of this concentration might reflect real exposure conditions. Mass balance cannot be calculated as only urinary and faecal excretions were measured, however total recovery rates of radioactivity were at least 94 % in all three groups tested for excretion. The results of this study can be considered as supplementary data.

Assessment and conclusion by RMS:
In line with the previous assessment (RAR, 2015) this study is considered supplemental due to the limitations of the study even without considering the laboratory where the test was conducted. The radioactivity was only investigated in the organs, tissues and carcass in one group receiving a low intravenous dose. Additionally, radioactivity in the bone was not determined. Also, the investigation of possible metabolites was only performed in the urine and not in faeces.
The data in the study confirms that absorption of the compound is low, and excretion fast.

B.6.1.1.9. Study 9

Data point	CA 5.1.1/010
Report author	[redacted]
Report year	1995
Report title	HR-001: Metabolism in the rat
Report No	[redacted] 332/951256
Document No	Not reported
Guidelines followed in study	Japanese MAFF, 59 NohSan, Notification No. 4200 (1985) OECD 417 US-EPA FIFRA 85-1
Deviations from current test guideline	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 to be acceptable.

Executive summary

A single oral dose of [^{14}C]-glyphosate (>98 % radiochemical purity) was administered by gavage, as aqueous solution (with addition of $NaHCO_3$ to achieve dissolution) at 10 or 600 mg/kg bw to male and female Sprague-Dawley rats to determine [^{14}C] plasma concentrations and plasma pharmacokinetic parameters (9 animals per sex), tissue distribution (6 animals per sex) and excretion (5 animals per sex) of the radio-labelled compound. Additionally, the magnitude and nature of metabolites has been investigated in urine and faeces.

The results showed that at least about a quarter (19 – 30 %) of the administered dose is absorbed from the gut. Absorption was similar for both sexes and at both dose levels, with peak plasma [^{14}C] concentrations observed around 3 - 6 h after dosing. The decline in plasma [^{14}C] concentration was monophasic with a half-life of about 8 h at 10 mg/kg bw and 5.9 h at 600 mg/kg bw dosing, indicating a rapid clearance. The areas under the plasma concentrations versus time curves (AUC_t) after the high dose level were approximately 100 to 120 fold higher compared to the low dose level. Neither dose- nor gender differences in excretion were noted. More than 90 % of the radioactivity was excreted with the urine and faeces within 48 h. About 65 – 77 % of the administered [^{14}C] are detected in faeces and 18 – 27 % was excreted via urine. Within 168 h more than 97 % of the administered dose was excreted via urine and faeces. About 74 – 84 % of the administered [^{14}C] are detected in faeces and 19 – 30 % is excreted via urine. No significant radioactivity was detected in exhaled air (<0.2 % of dose). The mean total radioactivity recovered within 168 h exceeded 95 %. The distribution of radioactivity in tissues was similar for males and females at both dose levels with maximum tissue level being detected within 3 h (high dose) or 6 h (low dose) and decreasing time-dependently afterwards. Highest concentrations were detected in the gastrointestinal tract and its content as well as stomach and its content. Concentrations in kidney, muscles and bones accounted for up to 0.79 % , 0.24 % and 0.12 % of the applied dose at the low dose and up to 1.00 %, 0.48 % and 0.09 % of the applied dose at the high dose, respectively at T_{max}. Concentrations in carcass accounted for up to 3.03 % of the applied dose at the low dose after 18 h and up to 2.85 % of the applied dose at the high dose after 3 h, respectively. Concentrations of radioactivity retained in the tissue 7 days post application were generally low (<0.4 % in carcass). Major urinary (18 – 27 %) and faecal (65 – 78 %) component was the parent compound. A minor compound (0.1 - 2.0 %) was identified as aminomethylphosphonic acid by TLC and HPLC co-chromatography. The nature of the other components (<2 %) was not investigated further.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	HR-001 (N-(phosphonomethyl)glycine)
Description:	solid
Lot/Batch #:	061221
Purity:	98.9 %
Stability of test compound:	Expiry date 1996-12-20

2. Radiolabelled test material:

Identification:	[^{14}C]-HR001
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	>98 % (TLC, followed by radioscanning)
Specific activity:	327.7 µCi/mg, 56 mCi/mmol
Stability of test compound:	Not reported

3. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	solid
Lot/Batch #:	09203L2
Purity:	99 %
Stability of test compound:	Not reported

4. Radiolabelled reference substance:

Identification:	[^{14}C]-Aminomethylphosphonic acid ([^{14}C]-AMPA)
Position of radiolabel:	Amino[^{14}C]methylphosphonic acid
Lot/Batch #:	Not reported
Radiochemical purity:	97.4 %
Specific activity:	2.0 GBq/mmol, 54 mCi/mmol
Stability of test compound:	Not reported

5. Vehicle and/ or positive control: Water, solubility was increased by addition of sodium hydrogen carbonate

6. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (CD)
Source:	[redacted]
Age:	6 - 8 weeks (males), 7 - 9 weeks (females)
Sex:	Males and females
Weight at dosing:	approximately 200 g
Acclimation period:	At least 5 days
Diet/Food:	Standard Laboratory Diet LAD 1 (Special Diet Services, Witham, Essex, UK), *ad libitum*

Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Individual housing in suspended, wire bottom, stainless steel cages After dosing: Excretion-balance experiments - individually in glass metabolism cages Blood/plasma kinetics - in stainless steel battery cages Tissue distribution - in stainless steel battery cages
Environmental conditions:	Temperature: 21 ± 2°C Humidity: 40 – 60 % Air changes: not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Preliminary excretion studies

In two independent experiments two rats (1 male, 1 female) received single oral doses of either 10 or 600 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine was collected at 0 - 6, 6 - 24 h, and every 24 h for 7 days in receivers cooled with solid CO_2. Faeces were collected every 24 h for 7 days. Expired air was passed through traps containing an ethanolamine/2-ethoxyethanol mixture (1:3, v/v). These traps were changed every 24 h for 7 days after dosing. The interior of the cages were washed with water at sacrifice after 7 days. Samples were analysed immediately or were stored at -20°C until taken for analysis.

Animal assignment and treatment: Excretion studies

In two independent experiments 10 rats (5 male, 5 female) received single oral doses of either 10 or 600 mg/kg bw by gavage and were placed in glass metabolism cages immediately thereafter. Urine and faeces were collected as described in the preliminary study. Blood was drawn by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement:
Adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Animal assignment and treatment: Plasma concentrations

In two independent experiments 18 rats (9 male, 9 female) received single oral doses of either 10 or 600 mg/kg bw by gavage. The animals were divided into three groups of six (3 per sex) and blood samples (0.5 mL) were taken from the tail vein into heparinised tubes at the following times from each group.
Group 1: prior to administration, 1, 4, 24 and 96 h
Group 2: 0.25, 2, 6, 48 and 120 h
Group 3: 0.5, 3, 12, 72 and 168 h
Each group was sacrificed upon completion of the specified sampling schedule.

Animal assignment and treatment: Quantitative tissue distribution

In two independent experiments 12 rats (6 male, 6 female) received single oral doses of 10 or 600 mg/kg /day by gavage. The animals were divided into two groups of six (3 per sex) and sacrificed by cervical dislocation 6 and 18 h (low dose) or 3 and 9 h (high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Data for 168 h (7 days) was provided by the excretion studies. Blood samples were taken by cardiac puncture (following light halothane anaesthesia) prior to sacrifice by cervical dislocation and plasma was obtained by centrifugation. The following tissues/organs were taken or sampled for radioactivity measurement: adrenals, bone, bone marrow (femur), brain, eyes, fat (abdominal), gastrointestinal tract, heart, kidneys, liver, lungs, lymph nodes (mesenteric), muscle (skeletal), ovaries, pancreas, pituitary gland, plasma, skin, spleen, stomach, testes, thymus, thyroid with parathyroid, urinary bladder, uterus and residual carcass. The contents of the gastrointestinal tract and stomach were analysed separately.

Dosing Formulation Analysis

The radiochemical concentration of the dosing preparation following formulation was determined by thin layer chromatography (TLC) and liquid scintillation counting.

Measurement of radioactivity

Faeces were initially extracted by homogenisation with chloroform:1N HCl (1:1, v/v) followed by further extracts with 1N HCl. After centrifugation radioactivity was measured in both extracts and residues. Samples of urine, plasma, solvent extracts contents of expired air traps, cage washings and other liquid samples were mixed with Special Scintillator MI-31 (Packard Instrument Co. Ltd, Reading, UK) for measurement of radioactivity by liquid scintillation counting (LSC). Samples of faecal residues, gastro-intestinal tract, liver, spleen and whole blood were combusted, absorbed, mixed with scintillation cocktail and analysed thereafter. Carcasses were digested for 48 h at 55°C in a solution of 2M NaOH in 30 % methanol containing Triton X-405 (10 % v/v). Tissue samples suitable for solubilisation were incubated at around 50°C for 18 - 24 h with solubiliser and mixed with scintillation cocktail and analysed thereafter. Radioactivity with less than twice background counts was considered to be below the limit of accurate quantification when performing LSC.

Isolation of the major urinary and faecal metabolites

Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by TLC or HPLC. Radiolabelled metabolites formed in the rat were identified by co-chromatographic comparison using different systems with the reference compound aminomethylphosphonic acid (AMPA) or [^{14}C] AMPA.

Thin layer chromatography (TLC)

TLC was carried out on pre-layered Merck cellulose F plates (0.2 mm, BDH Chemicals Ltd., Poole, UK) using the following development systems:

System 1: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid
(55:35:5:3.5:2, v/v/v/w/v)
System 2: Ethanol : water : ammonium hydroxide : trichloroacetic acid : acetic acid
(65:35:2.5:3.5:2, v/v/v/w/v)
System 5: Methanol : water : acetic acid
(67:33:1, v/v/v)

Radioactivity was detected with a Berthold Linear Analyser controlled by a computer system (Berthold Instruments (UK)) and proportions of radioactive components were measured by integrating the areas under the peaks on the radio chromatogram following subtraction of background levels. Alternatively, components were detected and quantified using a Fuji BAS 2000 Bioimage Analyser. The produced images of radioactive TLC plates were processed to generate quantitative data.

High performance liquid chromatography (HPLC)

Two HPLC methods were used. HPLC system 1 (gradient elution method; column: Sperisorp SAX HPLC column (Hichrom, UK) and guard column, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35) and HPLC

system 2 (isocratic method; column: glyphosate analytical column (BioRad, USA), eluent: 0.005 M KH_2PO_4 + 4 % methanol v/v, pH 2.1) were both linked to an UV- and a radio-detector. A Compaq Prolinea computer with Labchrom software was used to collect and process data from the UV and radio detectors. Samples were co-injected with a mixture of the reference standards. Fractions were collected and radio assayed by LSC.

Data evaluation

Data were quantified by LSC using models with automatic external Standard quench correction. After choosing the optimal channel setting, quench correction curves were prepared from radiochemical standards ([^{14}C]-hexadecane, Amersham International plc). The validity of the calibration curves was checked throughout the experiments. Samples with low levels of radioactivity were normally counted for 10 minutes or until the accumulated counts totalled 4 x 10^4.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

The preliminary study on two rats per dose (male/female) indicated that more than 90 % of the administered radioactivity was excreted within 7 days at the low and the high dose group after a single application of the test substance. Almost no radioactivity could be detected in expired air (about 0.15 %).

The main study with 10 rats per dose confirmed the initial observation, please refer to table given below. During the 7 days observation period 23 % and 19 % (male/female) were excreted in urine of the low dose group. Slightly higher percentages, 30 % and 29 % (male/female) of total administered radioactivity were detected in urine of the high dose group. The main portion of the radioactivity was detected at both dose levels within the first 48 h in males and females (21 % and 18 %, 10 mg/kg bw; 28 % and 27 %, 600 mg/kg bw). In both dose groups about 75 % of the administered radioactivity could be detected in the faeces of males and females within 7 days (75 % and 84 %, 10 mg/kg bw; 75 % and 74 %, 600 mg/kg bw). Again most of the radioactivity was detected within 48 h after dosing (72 % and 82 %, 10 mg/kg bw; 72 % and 69 %, 600 mg/kg bw). About 0.3 % of the radioactivity remained in the carcasses of the sacrificed animals after 7 days. Thus, in male and female rats almost all the administered radioactivity was excreted *via* the urine and faeces within 7 days (97 % and 104 % at 10 mg/kg bw; 105 % and 104 % at 600 mg/kg bw).

Table 6.1.1.9-18: HR-001: Metabolism in the rat [redacted] 1995): Excretion balance (in mean % of applied dose) at 168 h post dosing

Balance/Excretion	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Urine 0 - 6	2.63	3.25	11.55	9.08
Urine 6 - 24	15.85	12.69	13.85	13.36
Urine 24 - 48	2.82	2.41	2.33	4.40
Urine 48 - 72	0.54	0.44	0.59	1.07
Urine 72 - 96	0.24	0.19	0.30	0.40
Urine 96 - 120	0.15	0.13	0.21	0.24
Urine 120 - 144	0.09	0.07	0.17	0.17
Urine 144 - 168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal urine + cage wash	**22.51**	**19.37**	**30.26**	**29.50**
Faeces 0 - 24	60.28	74.59	58.94	46.28
Faeces 24 - 48	11.72	7.56	13.41	22.87
Faeces 48 - 72	1.18	1.34	1.36	3.83
Faeces 72 - 96	0.29	0.36	0.35	0.47
Faeces 96 - 120	0.17	0.27	0.36	0.23
Faeces 120 - 144	0.35	0.08	0.08	0.12
Faeces 144 - 168	0.64	0.10	0.15	0.35
Subtotal faeces	**74.63**	**84.30**	**74.65**	**74.15**
Carcass	0.33	0.27	0.31	0.39
Total	**97.47**	**103.94**	**105.22**	**104.04**

B. CONCENTRATION OF RADIOACTIVITY IN THE PLASMA

After a single oral dose of 10 mg/kg bw [^{14}C]-HR001 to rats mean peak concentrations of radioactivity in plasma occurred at 6 h and 2 h in males (0.22 µg equiv./mL) and females (0.28 µg equiv./mL), respectively. The absorption rate constants were 0.2963 h^{-1} in males and 0.4239 h^{-1} in females. Concentrations declined with an approximate half-life of 8.3 h in males and 7.8 h in females. The area under the concentration *versus* time curve (AUC_t) was 3.2 and 3.7 µg equiv./mL*h in males and females, respectively (please refer to the table below).

After a single oral dose of 600 mg/kg bw [^{14}C]- HR001 to rats mean peak concentrations of radioactivity in plasma occurred at 3 h in males (26 µg equiv./mL) and females (29 µg equiv./mL), respectively (please refer to the table below). The absorption rate constants were 0.2845 h^{-1} in males and 0.4477 h^{-1} in females. Thus absorption rate constants did not increase with dose. Concentrations declined with an approximate half-life of 5.9 h in males. The terminal half-life could not be calculated for females of the high dose group due to rapid clearance from plasma. The area under the concentration *versus* time curve (AUC_t) was calculated at 400 and 355 µg equiv./mL*h in males and females, respectively. These values were around 100 to 120 fold higher than the AUC_t obtained in the low dose group.
Measurements in whole blood in general lead to the same result.

Table 6.1.1.9-19: HR-001: Metabolism in the rat [redacted] 1995): Kinetic parameters in plasma after single oral dose of 10 or 600 mg/kg bw

	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
C_{max} [µg equiv./mL]	0.2219	0.2789	25.97	28.84
T_{max} [h]	6.00	2.00	3.00	3.00
AUC_t [µg equiv./mL×h]	3.20	3.70	399.90	355.30
AUC [µg equiv./mL×h]	3.80	4.20	419.00	*
Terminal rate constant [h^{-1}]	0.0840	0.0887	0.1174	*
Terminal halflife [h]	8.30	7.80	5.90	*

Absorption rate constant [h^{-1}]	0.2963	0.4239	0.2845	0.4477

* could not be calculated

AUC_t is the area under the plasma curve calculated up to the last detectable sample (up to 24 h within the present study)

AUC is the area under the plasma curve calculated to infinity

C. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Radioactivity in tissues was low at all times (please refer to tables below) and the relative tissue concentrations were comparable between sexes and dose groups. A time-dependent decrease of radioactivity was observed for all investigated tissues, except carcass where the highest concentration was detected after 18 h. The time dependent decrease and the overall low tissue concentration after 7 days do not indicate a potential of accumulation. Only the gastrointestinal tract (GIT), the stomach, the kidneys (the organs of excretion) the muscles and bones contained higher concentrations of radioactivity than the plasma. High levels of radioactivity were detected in the content of stomach and GIT. At 7 days p.a. the radioactivity in most tissues had decreased to around the limit of detection. Highest remaining concentrations were detected in carcass (<0.4 %).

Table 6.1.1.9-20: HR-001: Metabolism in the rat [redacted] 1995): Radioactivity in tissues after single oral dose of 10 mg/kg bw (in mean % of applied dose, except bone and skin expressed as % of applied dose/g)

Tissue	Males			Females		
	6 h (n=3)	18 h (n=3)	168 h (n=5)	6 h (n=3)	18 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.12	0.10	0.02	0.10	0.09	0.03
Bone marrow	0.01	0.01	<0.01	0.01	0.01	0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.06	0.04	0.01	0.04	0.03	0.01
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
GIT contents	31.56	4.89	0.01	34.54	14.30	0.01
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	0.79	0.36	<0.01	0.67	0.26	<0.01
Liver	0.07	0.09	0.01	0.06	0.07	0.01
Lungs	0.01	0.01	<0.01	0.01	0.01	<0.01
Lymph nodes	0.09	0.05	<0.01	0.04	0.04	<0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	<0.03
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.02	<0.01	<0.01	0.01	<0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.12	0.03	<0.01	0.13	0.03	<0.01
Skin	0.01	0.01	<0.01	0.01	0.01	<0.01
Spleen	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.47	0.60	0.60	2.56	0.62	<0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.20	0.04	<0.03	0.15	0.05	<0.03

Table 6.1.1.9-21: HR-001: Metabolism in the rat [redacted] 1995): Radioactivity in tissues after single oral dose of 600 mg/kg bw (in mean % of applied dose, except bone and skin expressed as mean % of applied dose/g)

Tissue	Males			Females		
	3 h (n=3)	9 h (n=3)	168 h (n=5)	3 h (n=3)	9 h (n=3)	168 h (n=5)
Adrenal glands	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Bone	0.09	0.10	0.02	0.09	0.05	0.02
Bone marrow	0.01	0.01	<0.01	0.01	0.01	<0.01
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 6.1.1.9-21: HR-001: Metabolism in the rat [redacted] 1995): Radioactivity in tissues after single oral dose of 600 mg/kg bw (in mean % of applied dose, except bone and skin expressed as mean % of applied dose/g)

Tissue	Males			Females		
	3 h (n=3)	9 h (n=3)	168 h (n=5)	3 h (n=3)	9 h (n=3)	168 h (n=5)
Carcass	1.87	1.70	0.31	2.85	2.41	0.39
Eyes	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fat (abdominal)	0.09	0.05	<0.01	0.09	0.02	<0.01
Gastrointestinal tract	19.71	9.99	0.01	20.90	9.33	0.01
GIT contents	30.48	13.19	0.02	22.65	12.86	0.03
Heart	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Kidneys	1.00	0.55	<0.01	0.82	0.21	<0.01
Liver	0.06	0.14	0.01	0.07	0.06	0.02
Lungs	0.02	0.01	<0.01	0.02	0.01	<0.01
Lymph nodes	0.07	0.02	<0.01	0.04	0.01	<0.01
Muscle (skeletal)	0.38	0.19	<0.05	0.48	0.18	<0.05
Ovaries	-	-	-	<0.01	<0.01	<0.01
Pancreas	0.01	0.01	<0.01	0.01	0.01	<0.01
Pituitary gland	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Plasma	0.26	0.07	<0.01	0.30	<0.01	<0.01
Skin	0.01	<0.01	<0.01	0.01	<0.01	<0.01
Spleen	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Stomach	3.53	3.36	<0.01	4.33	3.14	<0.01
Stomach contents	28.73	32.70	0.02	34.20	45.01	0.02
Testes	0.01	0.01	<0.01	-	-	-
Thymus	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Urinary bladder	0.01	0.01	<0.01	<0.01	<0.01	<0.01
Uterus	-	-	-	0.01	<0.01	<0.01
Whole blood	0.28	0.08	0.01	0.30	0.05	<0.01

D. PROPORTION OF RADIOACTIVE COMPONENTS IN URINE

Major urinary (18 - 27 %) and faecal (65 - 78 %) component was the parent compound. One further minor component was also observed in urine (0.1 - 0.3 %) and identified as aminomethylphosphonic acid by TLC and HPLC co-chromatography. In faeces two further minor components were detected (1 - 2 %, low dose; 0.3 - 0.6 %, high dose) one of them could be identified as aminomethylphosphonic acid by TLC and HPLC co-chromatography. The nature of the other component (<2 %) was not investigated further.

E. RECOVERY OF RADIOACTIVITY

The total mean radioactivity recovered within 168 h exceeded 95 % and was below 106 %.

III. CONCLUSIONS

After oral administration of glyphosate at least 20 % are absorbed as indicated by urinary excretion. Absorption was independent of sex but the areas under the plasma concentration vs time curves (AUC_i) after the high dose were increased by 100 to 120 fold compared to the low dose. Only the gastrointestinal tract, the stomach, the kidneys (the organs of excretion) the muscles and bones contained higher concentrations of radioactivity than the plasma. About 75 % and 25 % of the parent compound was excreted via faeces and urine, respectively. Additionally, aminomethylphosphonic acid was detected in faeces and urine accounting for ≤1.6 % of the dose. The nature of the other compounds in faeces (<2 %) was not investigated further. Elimination from the body was fast with half-life´s of 6 - 8 h at the low and high dose, respectively. After 168 h the highest remaining concentrations was detected in carcass (<0.4 % of applied dose). The time dependent decrease and the overall low tissue concentration after 7 days do not indicate a potential of accumulation.

Assessment and conclusion by applicant:

After single oral administration of 10 or 600 mg/kg bw glyphosate to both male and female rats at least 20 % was absorbed (based on the amount excreted *via* urine). About 75 % and 25 % of the dose was excreted *via* faeces and urine, respectively, mainly as the unchanged parent compound within 7 days after administration. Additionally, aminomethylphosphonic acid was detected in faeces and urine accounting for ≤1.6 % of the dose. The nature of the other components in faeces (<2 %) was not investigated further.

Investigation of pharmacokinetics in plasma revealed that plasma peak levels were reached 6 h (males) and 2 h (females) after administration of the low dose and 3 h (males and females) after administration of the high dose. No further significant gender- or dose-dependent differences were noted.

Tissue concentrations were overall low at all investigated sampling time points and dose levels, except the GI tract, stomach and contents. Further, muscles, bones and the kidneys showed higher levels than plasma accounting for up to 0.79 % (low dose) and 1.00 % (high dose) at T_{max}. Thereafter, radioactivity levels decreased time-dependently. Albeit repeated dosing was not investigated within the present study, the findings do not indicate an accumulation, which is also supported by observations made within other studies.

Assessment and conclusion by RMS:

Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Almost complete excretion within 7 days, with most being excreted within 24 hours Peak plasma concentrations are reached approximately 2-6 hours post dosing and the absorption rate does not increase with increased dose. Absorption is slightly faster in female animals.
This study confirms the tissue distribution of the previously discussed studies.
Glyphosate is mainly excreted unmetabolized. A minor metabolite in both urine and faeces is AMPA. The identity of a second minor metabolite is not investigated.
This conclusion is in line with the previous assessment (RAR, 2015).

B.6.1.1.10. Study 10

Data point	CA 5.1.1/011
Report author	[redacted]
Report year	1992
Report title	[^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study
Report No	6365-676/1
Document No	Not reported
Guidelines followed in study	US-EPA FIFRA 85-1
Deviations from current test guideline	Yes, limited focus (only 3 male animals at a single dose level)
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered to be reliable with restrictions and acceptable as preliminary study only.

Executive summary

The objectives of this preliminary study was to determine the pharmacokinetics and tissue distribution of radioactivity in three male rats after a single oral dose of [^{14}C]-glyphosate using blood sampling and whole-body autoradiography, respectively. The aim was to ascertain appropriate blood sampling times and identify the distribution of radioactivity for a definitive adsorption, distribution, metabolism, and excretion study.

Overnight fasted male rats were orally administered with [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw. Following administration to one group of three rats, blood samples were collected at regular intervals up to 48 hour post-dose and radioactivity determined in plasma. After administration to a second group of 3 rats, whole-body autoradiography was performed on one animal at 4, 10 and 24 h post-dosing and target tissues identified.

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations (C_{max}) reached within 4 h was 1.769, 1.137, and 0.705 µg equiv/mL for three animals. Thereafter plasma levels decreased exponentially to non-detectable levels at 12 h post-dose. The elimination half-lives were 6.196 h and 12.35 h for two animals. A value could not be obtained for the third animal.

Whole-body autoradiography showed that distribution of radioactivity into tissues is limited. The greatest concentrations of radioactivity were detected 10 h after application. The highest concentrations of radioactivity were associated with bone and bone marrow, cartilage, some parts of the gastro-intestinal tract, kidney and urinary tract and the nasal mucosa. Except for radioactivity in bone and bone marrow, which appear to be possible target tissues for this compound, there were negligible tissue concentrations 24 h post-dose.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	White crystalline solid
Lot/Batch #:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	Non-sensitive to sunlight; No degradation observed in 14 days accelerated storage stability test at 54 ± 2° C (CIPAC method MT 46.1.1)

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	CFA 745 C4
Radiochemical purity:	The stated radiochemical purity was 97.4 %, however, subsequent to this investigation this was amended to 94.3 %
Specific activity:	307.7 µCi/mg (11.38 MBq/mg)
Stability of test compound:	Stored at -20° C and stability in formulation assessed

3. Vehicle and/ or positive control: 0.9 % w/v sodium chloride

4. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	Approximatively 9 weeks old on arrival
Sex:	Male
Weight at dosing:	249 - 275 g at the start of treatment for the pharmacokinetic study and 267 - 296 g at the start of the autoradiography study
Acclimation period:	Approximately 10 days
Diet/Food:	During acclimatisation: Commercial pellet diet, SQC Rat and Mouse Maintenance Diet No 1, Expanded(Special Diet Services, Stepfield, Witham, Essex), *ad libitum* Before experiments: The diet was removed for approximately 14 h before and 4 h after administration of the radiolabelled test article.
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of up to five per cage, in wire floor polypropylene cages suspended over polypropylene dirt trays containing soft white wood flakes. Experimental conditions: Following administration of [^{14}C]-glyphosate the three animals from dose Group A were transferred to grid floor cage.
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 40 - 70 % Air changes: 15/h 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment: Pharmacokinetic study

Rats were randomly allocated unique numbers using ear tags and/or tail marking as shown below:

Study type	Animal identification	Group name
Pharmacokinetic	118M, 119M, 121M	A

Each animal received a single oral administration of [^{14}C]-glyphosate at a dose level of 30 mg/kg bw corresponding to a nominal radioactive dose of 15 µCi per animal. Following administration of [^{14}C]-glyphosate to three rats (Group A), blood samples (approximately 100 µL) were collected from the lateral tail vein pre-dose and at 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24 and 48 h after treatment and the radioactivity determined in plasma. The animals were sacrificed in a rising concentration of carbon dioxide.

Animal assignment and treatment: Distribution study

Rats were randomly allocated unique numbers using ear tags and/or tail marking as shown below:

Study type	Animal identification	Group name
Whole-body autoradiography	2M, 3M, 4M	B

Each animal received a single oral administration of [^{14}C]-glyphosate at a dose level of 30 mg/kg bw corresponding to a nominal radioactive dose of 30 µCi per animal. Following administration of [^{14}C]-glyphosate to three male rats, one male per time point was sacrificed 4, 10 and 24 h after treatment for whole-body autoradiography.
After sacrifice, each carcass was pinned out on a board and rapidly and embedded in a block of aqueous carboxymethylcellulose (2 % w/v) and mounted onto the stage of a PMV 450MP cryo-microtome maintained at about –20 °C. Sagittal sections (about 25 µm) were then obtained at 6 levels through the carcass:

Level A; Exorbital lachrymal gland
Level B: Intra-orbital lachrymal gland
Level C: Harderian gland
Level D: Adrenal gland
Level E: Thyroid
Level F: Brain and spinal cord

Autoradiograms were prepared by contact with autoradiographic film for 108 days before fixing and washing.

Dosing Formulation Analysis

The stability of the formulation was not determined due to difficulties with the reproduction of the Thin Layer Chromatography.

Measurement of radioactivity

Portions of plasma were added directly to liquid scintillant and subjected to Liquid Scintillation Counting (LSC). All radioassays were performed in duplicate. Radioactivity was measured for 10 min or for 2 sigma % using a Beckman or Packard Tri-Carb liquid scintillation counter with facilities for computing quench-corrected disintegrations per minute (dpm).

The limit of detection for each batch of samples analysed by direct counting is taken as 2 times the mean background disintegration rate obtained from vials containing an equivalent volume of an appropriate solvent in liquid scintillant.

Thin layer chromatography (TLC)

TLC was carried out on cellulose plates (500 µm) using the following development systems to assess the stability of the formulated [^{14}C]-glyphosate upon preparation and after storage at room temperature for 4 h:
System 1: Methanol : water (67:33 v/v)
System 2: Ethanol : water : 15N ammonium hydroxide : trichloroacetic acid : acetic acid
(55:35:2.5:35:2 , v/v/v/w/v)
The plates were developed for at least 18 cm and visualised by 0.5 % ninhydrin in butanol then heated at 100ºC for 5 minutes. Radioactivity was quantified with a linear analyser.

Data evaluation

Liquid scintillation counts and weighing data were captured on-line using a validated data acquisition system. The limit of detection for each batch of samples analysed by direct LSC is taken as 2 times the mean background disintegration rate obtained from vials containing an equivalent volume of an appropriate solvent in liquid scintillant.

II. RESULTS AND DISCUSSION

A. PHARMACOKINETIC STUDY

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations of 1.769, 1.137 and 0.705 µg equivalent of [^{14}C]-glyphosate/mL were reached within 3 - 4 h. Thereafter plasma levels declined exponentially to non-detectable levels 12 h after treatment. The elimination half-lives were 6.2 and 12.4 h for two animals. A value could not be obtained for the third animal. Please refer to the tables below.

Table 6.1.1.10-22: [^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study [redacted] 1992): Pharmacokinetic parameters for plasma radioactivity in rats following a single oral administration of [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw

Animal no. and sex	Cmax (µg eq/mL)	Tmax (h)	AUC (µg eq.h/mL)	$t_{1/2}$ (h)
118M	1.769	3	18.62	6.2
119M	1.137	4	23.09	12.4
121M	0.705	4	N/A	N/A

N/A not applicable

Table 6.1.1.10-23: [^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study [redacted] 1992): Plasma concentrations of radioactivity in male rats following a single oral administration of [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw

Time (h)	µg equivalents of [^{14}C]-glyphosate/mL of plasma		
	118M	119M	121M
Pre-dose	ND	ND	ND
0.5	ND	ND	ND
1	0.640	0.626	ND
1.5	1.137	0.823	ND
2	1.356	1.032	0.515
2.5	1.671	0.887	0.580
3	1.769	1.118	0.695
4	1.565	1.137	0.705
6	1.170	0.928	0.612
9	0.910	0.849	0.649
12	ND	ND	ND
24	ND	ND	ND
48	ND	ND	ND

ND not detected

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Whole-body autoradiography showed that distribution of radioactivity into tissues was limited. The greatest concentrations of radioactivity were detected 10 h after treatment. The highest concentrations of radioactivity were associated with bone and bone marrow, cartilage, some parts of the gastrointestinal tract, kidney and urinary tract and the nasal mucosa. Except for radioactivity in bone and bone marrow, there were negligible tissue concentrations 24 h post-dose. Please refer to the table below.

Table 6.1.1.10-24: [^{14}C]-Glyphosate: Absorption and distribution in the rat – preliminary study [redacted] 1992): Distribution of relative levels of radioactivity in the tissues of male rats as revealed by whole-body autoradiography following a single oral administration of [^{14}C]-glycosate at a nominal dose level of 30 mg/kg bw

Tissue type	Tissue	Animal number and time of sacrifice		
		4M	2M	3M
		4 h	10 h	24 h
Bone	Bone marrow	2	2	2
	Bone	2	2	2
	Ischium	2	2	2
	Ileum	2	2	2
	Jaw bone	2	2	2
	Rib	2	2	2
	Sternum	2	2	2
	Vertebrae	2	2	2
Connective	Cartilage	1	2	0
	Epimysium	0	1	0
	Fascia	0	1	0
	Xiphoid cartilage	NP	2	NP
Endocrine	Adrenal	0	1	0
	Pituitary	0	1	0
	Thymus	0	1	0
	Thyroid	1	1	1
Secretory	Exorbital lachrymal gland	0	1	0
	Intra-orbital lachrymal gland	0	1	0
	Salivary glands	1	1	1
Gastrointestinal	Caecal contents	3	3	3
	Caecal mucosa	3	3	3
	Large intestine contents	3	3	3
	Large intestine mucosa	3	1	3
	Small intestine contents	2	3	1
	Small intestine mucosa	3	3	3
	Stomach contents	0	1	0
	Stomach mucosa	2	3	2
Gonads	Prostate	0	1	0
	Seminal vesicles	0	1	0
	Testis	1	1	0
Muscular	Myocardium	0	1	0
	Tongue	1	1	0
Urinary	Bladder	3	3	2
	Kidney cortex	3	3	2
	Kidney medulla	1	2	1
Others	Blood	1	1	0
	Liver	1	1	1
	Lung	1	1	1

	Nasal mucosa	2	3	1
	Skin	1	1	1
	Spleen	1	1	0
	Trachea	1	1	0

NP Organ/tissue not present on autoradiogram.
The assessment of the relative levels of radioactivity in the autoradiograms was made by visual inspection of the autoradiograms whilst viewing them on a standard X-ray viewing box. The relative levels of radioactivity in the tissues were described as 3 ="high", 2 = "moderate", 1 = "low", 0 = radioactivity not detected.

III. CONCLUSIONS

Following oral administration of [^{14}C]-glyphosate, systemic absorption of radioactivity was poor and tissue distribution of [^{14}C]-glyphosate and/or its radiolabelled metabolites were limited.

Assessment and conclusion by applicant:

The plasma profiles and distribution of radioactivity have been studied in a pilot absorption and distribution study using three male rats with the aim to obtain preliminary data for a subsequently following full-range ADME study.

Following a single oral administration of [^{14}C]-glyphosate at a nominal dose level of 30 mg/kg bw low levels of radioactivity were detected in plasma. Maximum plasma concentrations (C_{max}) between 0.7 and 1.8 µg equiv/mL were reached within 4 hours (T_{max}) No radioactivity was detectable in plasma 12 h after treatment. The elimination half-lives were 6.2 and 12.4 h for the two animals for which these values could be obtained.

Whole-body autoradiography revealed a limited tissue distribution of radioactivity. Highest levels were observed 10 h post-dose with a specific accumulation in bone, especially in the growing regions, i.e. the epiphyses. This affinity may be attributed to the phosphonomethyl moiety of glyphosate. Except for radioactivity in bone and bone marrow, negligible tissue concentrations were noted 24 h post dose.

The results of this study can be considered as supplemental data only.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant that the study is preliminary and should be considered as supplemental data only. The results confirm the outcome of the previously discussed studies.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.11. Study 11

Data point	CA 5.1.1/012
Report author	[redacted]
Report year	1992
Report title	[^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat
Report No	7006-676/2
Document No	Not reported
Guidelines followed in study	US-EPA FIFRA 85-1
Deviations from current test guideline	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 to be acceptable.

Executive summary

The absorption, distribution, metabolism and excretion of oral and intravenously administered [^{14}C]-glyphosate have been investigated in male and female Sprague Dawley rats after a single intravenous and oral dose of 30 mg/kg bw, a single oral dose of 1000 mg/kg bw and after 15 daily oral doses of 30 mg/kg bw/day. Samples were collected for a period of 7 days after (last) administration. Radioactive tissue residues were determined after at least 90 % of the dose had been excreted. Radioactivity in excreta was characterised by chromatographic spectroscopic methods.

The recovery of administered radioactivity in excreta exceeded 90 % in all investigations. The proportion of radioactive dose absorbed from the gut, estimated by comparison of renal elimination following single intravenous or oral administration at 30 mg/kg bw, was 34 % (males) and 36 % (females) respectively. Absorption was similar for both sexes and at all tested doses.

The radioactive tissue residues present after >90 % of the dose had been eliminated were low (<1.5 % of dose) irrespective of dosing regimen. Highest residues were present in bone. The distribution of radioactivity in tissues was similar for males and females after both dose levels.

No radiolabelled metabolites of [^{14}C]-glyphosate were found in any of the investigations. The identity of the radioactive component as glyphosate was confirmed by FT-IR spectrometer.

After intravenous dosing, elimination in urine accounted for ca. 85 % of the dose compared with <4 % in faeces. After oral dosing about 47 - 60 % of the administered [^{14}C]-glyphosate were detected in faeces and 23 - 35 % in urine. Renal elimination was more protracted following oral administration of [^{14}C]-glyphosate continuing for at least 24 h post-dose. At least 80 % of the radioactivity was excreted with the urine, faeces and exhaled air within 48 h. No significant radioactivity was detected in exhaled air (<0.1 % of dose). There was neither a noticeable difference in excretion in males and females nor at different dose levels.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification:	Glyphosate (N-(phosphonomethyl)glycine)
Description:	White crystalline solid
Lot/Batch #:	206-JaK-25-1
Purity:	98.6 %
Stability of test compound:	Non-sensitive to sunlight; No degradation observed in 14 days accelerated storage stability test at 54 ± 2°C (CIPAC method MT 46.1.1)

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	CFQ.6228
Radiochemical purity:	>98 % by two TLC systems (97 % by HPLC)
Specific activity:	52 mCi/mmol, 1.0 mCi/mL
Stability of test compound:	The stability was assessed by two TLC systems.

3. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

4. Vehicle and/ or positive control: 0.9 % w/v sodium chloride in water

5. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	6 - 10 weeks on arrival
Sex:	Males and females, nulliparous and non-pregnant
Weight at dosing:	150 - 200 g on arrival in May 1990
Acclimation period:	At least 7 days
Diet/Food:	Commercial pellet diet , SQC Rat and Mouse Maintenance Diet No 1, Expanded (Special Diet Services, Stepfield, Witham, Essex), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Groups of five animals per cage according to sex, in wire floor polypropylene cages suspended over polypropylene dirt trays containing soft white wood sawdust. After dosing: Individually in glass metabolism cages suitable for the separate collection of urine, faeces and expired air.
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 40 - 70 % Air changes: At least 10/hour 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment

Group	Route of dosing	Frequency of dosing	Dose level (mg/kg bw)		Radioactive dose (µCi)
A	Intravenous	Single	30	Low	25
B	Oral	Single	30	Low	50
C	Oral	Multiple*	30	Low	25
D	Oral	Single	1000	High	25

*14 consecutive daily doses of glyphosate followed approximately 24 h later by radiolabelled glyphosate

Following administration of [^{14}C]-glyphosate (1st treatment in May 1990), rats from each dose group (5 male, 5 female,) were placed in individual glass metabolism cages. Cage side observations were performed in the morning.

Urine and faeces were collected at the following time intervals: 0 - 4, 4 - 8, 8 - 12, 12 - 24, 24 - 36, 36 - 48, 48 - 72, 72 - 96, 120 - 144, 144 - 168 h after treatment.

Expired air was trapped in 2-ethoxyethanol : ethanol amine (3:1 by volume) and was sampled as per urine collections until it was determined that less than 1 % of the administered radioactivity was present.

After each collection of excreta, cage debris was removed and cages washed with water. At the end of the collection period, cages were washed thoroughly with water and then methanol. The radioactivity present in urine, faeces, cage washings, cage debris and expired air was determined. As soon as a mean of >90 % of the administered radioactivity was excreted or after a maximum of 168 h, the rats were exsanguinated under halothane anaesthesia.

The following tissues were removed or sampled and assayed for radioactivity: blood, bone (femur), bone marrow, brain, fat (abdominal), heart, kidney, 1iver, lungs, muscle (skeletal), ovaries/testes, plasma, salivary gland, spleen, uterus, residual carcass.

Dosing Formulation Analysis

The stability of the formulated [^{14}C]-glyphosate dissolved in 0.9 % saline (at approx. 6 mg/mL) was assessed by two Thin layer chromatography (TLC) systems at 0, 1, 2, 10 and 16 days post-formulation. Comparison of the respective chromatograms showed no significant degradation of [^{14}C]-glyphosate over this time. The specific radioactivity of the [^{14}C]-labelled test substance prior to formulation and the homogeneity of the dosing preparation was determined by liquid scintillation counting.

Measurement of radioactivity

Portions of faeces, cage debris, bone, blood, spleen and liver were combusted. Combusted products were absorbed in Carbo-Sorb™ mixed with Permafluor® V and the radioactivity determined by liquid scintillation counting. Combustion and trapping efficiencies were found to be in excess of 96 %. All other samples were solubilised with Soluene-350™ or Soluene-100™ and incubated as necessary. Hionic-Fluor was admixed and, following dark adaption overnight, the samples were assayed for radioactivity using a liquid scintillation counter. All radioassays were performed in duplicate. Radioactivity was measured for 10 min or for 2 sigma % using a Beckman (Beckman, High Wycombe, Bucks) or Packard Tri-Carb liquid scintillation counter (Canberra Packard, Pangbourne, Berks) with facilities for computing quench-corrected disintegrations per minute (dpm).

Isolation of the major urinary and faecal metabolites

The metabolite profiles of urine and faeces samples from dose Groups A to D were investigated using one HPLC and two TLC systems. They were quantitatively examined for glyphosate and the potential metabolite, AMPA.

Thin layer chromatography (TLC)

TLC was carried out on Merck cellulose plates (0.5 mm) using an 18 cm development system and the following solvent systems:

System 1: Methanol : water (67:33 by volume);
System 2: Ethanol : trichloroacetic acid (10 % aqueous solution) : ammonium hydroxide (20 M) : acetic acid (17M), (55:35:2.5:2 by volume).

Glyphosate and aminomethyl phosphonic acid (AMPA) reference standards were visualized with ninhydrin spray reagent.

Radioactivity was detected with a Raytest RITA Linear Analyser (Lablogic systems Ltd.) and autoradiography Hyperfilm ß-max (Amersham International plc). Regions of interest were located by reference to an autoradiogram. They were assigned manually and the percent radioactivity associated with each region determined using the RITA data system.

High performance liquid chromatography (HPLC) and Spectrometry

HPLC system (gradient elution method; column: Merck Lichrosorb RP-18, Solvent A: 95 % HPLC phosphate buffer, 5 % acetonitrile, Solvent B: 15 % HPLC phosphate buffer, 85 % acetonitrile linked to a Fluorescence

detector and on-line radioactivity monitor. Reference standards were glyphosate and aminomethyl phosphonic acid (AMPA).

Extracts from dose Group D, pooled male urine (4 - 8 h post dose) and pooled male faeces (12 - 24 h post dose) were analysed using TLC solvent System 1. Each sample was applied to the origin of a separate TLC plate as a thin band (~ 18 cm long). Immediately after development, the location of the major region of radioactivity was determined using a RITA linear analyser to scan several positions across each plate. The cellulose layer containing this region was scraped from the plate and radioactivity extracted into methanol. Solutions of non-labelled and [^{14}C]-glyphosate standard in methanol (~ 0.1 mg/mL) were prepared. The standards and extracted samples were analysed using a Mattson 2020 Galaxy FT- IR spectrometer (Mattson Instruments Ltd).

Data evaluation

Liquid scintillation counts and weighing data were either entered manually or captured on-line using a validated data acquisition system. The limit of detection for the analysis sample from the excretion balance study was taken as twice the mean background from measured blank samples of the same type.

II. RESULTS AND DISCUSSION

A. CAGE SIDE OBSERVATIONS

No overt pharmacological or toxicological signs were observed in the test animals which could have been attributed to the administration of [^{14}C]-glyphosate.

B. EXCRETION AND RETENTION OF RADIOACTIVITY

More than 90 % of the administered radioactivity was recovered within 7 days by the low and the high dose groups after a single oral or intravenous application of the test substance. After multiple oral dosing 90 % of the administered radioactivity was recovered.

Single bolus intravenous dose at 30 mg/kg bw:
The major proportion of radioactivity excreted in urine (86.0 % male; 84.2 % female) was within the first 4 h (72.1 % male; 71.9 % female). Considering cage wash to calculate the total urinary excretion 92.9 % and 97.4 % were excreted in urine by males and females, respectively.

Faecal excretion of radioactivity was 3.4 % in male and 1.5 % in females. Less than 0.03 % was eliminated in expired air. At termination (168h), radioactive residues were low (1.35 % male; 1.09 % female). Of the tissues sampled at termination, bone contained the highest concentration (4.2 µg equiv/g, male; 4.4 µg equiv/g, female), all remaining tissues possessing <0.5 µg equiv/g except for female bone marrow (1.3 µg equiv/g). Fat, muscle and testes were devoid of quantifiable quantities of radioactivity.

Single oral dose at 30 mg/kg bw:
The experiment was terminated after 120 h since the total excreted exceeded 90 % at that time point. The major proportion of radioactivity excreted in faeces (58.8 % male; 56.5 %, female) was within 24 h post dose (45.8 %, males; 40.7 %, females). Urine accounted for 29.0 % in males and 30.7 % in females with significant amounts still excreted at 24 h post-dose (7.1 %, males; 7.2 %, females). Less than 0.1 % was eliminated in expired air. Residual radioactivity in tissues and carcass at 120 h post-dose accounted for about 0.6 % (males and females). Concentrations in bone were 2.3 µg equiv/g in males and 2.6 µg equiv/g in females. The remaining tissues excised contained <0.6 µg equiv/g; fat (males only), muscle and testes were devoid of quantifiable quantities of radioactivity.

Single oral dose at 1000 mg/kg bw:
The major proportion of radioactivity excreted in faeces (53.3 % male; 60.4 % female) was within 24 h post-dose (38.6 %, males; 48.3 %, females). Urine accounted for 30.6 % in males and 22.4 % in females. Pulmonary elimination was negligible. At 168 h post-dose, radioactive residues in tissues and carcass were low (0.5 %, males; 0.4 %, females). Bone contained the highest concentrations of radioactivity (56.3 µg equiv/g, males; 40.7 µg equiv/g, females), some 25- and 15-fold the levels observed after the single oral dose for a 33-fold increase in dose. The organs of metabolism and excretion contained ca. 5 - 6 µg equiv/g in male animals, whereas in females, though the level in the kidney was ca. 4 µg equiv/g, the liver contained no detectable radioactivity. With the exception of the lungs and bone marrow, no quantifiable radioactivity was determined in the remaining tissues.

14 days repeated oral doses at 30 mg/kg bw:
The experiment was terminated after 72 h since the total excreted exceeded 90 % at that time point. Most radioactivity was excreted in faeces (49.6 %, males; 46.7 %, females), the majority within 24 h post-dose. Urine accounted for 34.28 % (males) and 34.63 % (females) of the dose. The elimination was slower than after the intravenous administration, with 5.1 % (males) and 6.2 % (females) still excreted at 12 - 24 h post-dose. In both sexes, expired air accounted for <0.1 % of the administered dose. The investigation was terminated ca. 72 h post-dose as a mean of ca 90 % of dose had been recovered. At this time, residual radioactivity in tissues and the remaining carcass accounted for 0.96 % (males) and 0.83 % (females) of the dose. As with the single doses, bone exhibited the highest concentration of radioactivity (3.1 µg equiv/g, males; 2.5 µg equiv/g, females), all other tissues contained <0.6 µg equiv/g. Fat, muscle, testes and brain (females only) contained no quantifiable radioactivity.

ble 6.1.1.11-25: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat [redacted] 1992): Mean (±SD) recovery of radioactivity (% of applied dose) following administration of [^{14}C]-glyphosate to the rat (at termination)

Sample	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Urine	85.98	84.18	29.04	30.71	34.28	34.63	30.55	22.41
	(6.286)	(8.127)	(8.280)	(2.772)	(10.37)	(5.994)	(5.369)	(6.612)
Cage wash	6.948	13.25	6.884	7.453	5.145	7.324	12.08	16.66
	(4.549)	(6.399)	(2.914)	(3.165)	(2.025)	(1.832)	(7.297)	(8.328)
Subtotal urine + cage wash	92.928	97.43	35.924	38.163	39.425	39.775	42.63	39.07
Faeces	3.422	1.484	58.84	56.53	49.64	46.73	53.27	60.37
	(3.150)	(0.811)	(7.989)	(4.635)	(10.99)	(5.558)	(8.928)	(10.30)
Expired air (CO_2 Trap 1)	0.014	0.023	0.075	0.065	0.085	0.055	0.064	0.067
	(0.013)	(0.013)	(0.027)	(0.022)	(0.064)	(0.015)	(0.012)	(0.018)
Expired air (CO_2 Trap 2)	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.006
	(0.013)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.013)
Cage debris	0.025	0.021	1.160	1.315	0.033	0.389	3.268	0.519
	(0.036)	(0.029)	(1.188)	(1.192)	(0.061)	(0.519)	(5.628)	(0.682)
Subtotal	**96.397**	**98.907**	**96.011**	**96.075**	**89.185**	**89.125**	**99.231**	**100.0**
Tissues***	1.353	1.093	0.619	0.635	0.955	0.825	0.469	0.400
	(0.604)	(0.169)	(0.132)	(0.108)	(0.210)	(0.171)	(0.052)	(0.076)
Total (Mass Balance)	**97.75**	**100.0**	**96.63**	**96.71**	**90.14**	**89.95**	**99.70**	**100.4**

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
*** Carcass plus whole tissues

Table 6.1.1.11-26: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat [redacted] [redacted] 1992): Excretion balance (in mean % of applied dose) at termination

Balance/Excretion	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Urine 0 - 4	72.100	71.89	3.215	3.150	8.992	5.515	5.561	2.078
Urine 4 - 8	7.344	6.397	9.638	11.91	11.79	15.21	14.21	13.32
Urine 8 - 12	2.442	2.056	4.239	4.489	4.950	5.006	2.918	2.386
Urine 12 - 24	1.694	1.429	7.137	7.202	5.091	6.184	3.819	2.622
Urine 24 - 36	0.813	0.778	3.163	2.266	2.387	1.520	1.991	0.966
Urine 36 - 48	0.369	0.364	0.752	0.775	0.622	0.427	0.806	0.378
Urine 0 - 48h	**84.762**	**82.914**	**28.144**	**29.792**	**33.832**	**33.862**	**29.305**	**21.750**
Urine 48 - 72	0.458	0.497	0.588	0.551	0.447	0.766	0.687	0.384
Urine 72 - 96	0.320	0.309	0.197	0.238	NS	NS	0.247	0.165
Urine 96 - 120	0.187	0.206	0.115	0.134	NS	NS	0.172	0.096
Urine 120 - 144	0.141	0.145	NS	NS	NS	NS	0.096	0.012
Urine 144 - 168	0.112	0.109	NS	NS	NS	NS	0.043	0.000
Cage wash 0 - 4	4.829	10.97	0.767	1.581	1.403	1.065	1.152	4.800
Cage wash 4 - 8	1.279	1.459	1.296	2.618	0.383	1.712	3.531	5.243
Cage wash 8 - 12	0.363	0.298	2.452	1.173	0.293	0.868	4.453	4.262
Cage wash 12 - 24	0.139	0.122	1.557	1.449	0.435	2.383	1.767	1.681
Cage wash 24 - 36	0.071	0.100	0.539	0.284	0.269	0.716	0.549	0.333
Cage wash 36 - 48	0.088	0.092	0.165	0.222	0.109	0.376	0.201	0.155
Cage wash 48 - 72	0.062	0.106	0.055	0.070	0.253	0.203	0.179	0.101
Cage wash 72 - 96	0.037	0.040	0.027	0.030	NS	NS	0.129	0.055
Cage wash 96 - 120	0.035	0.026	0.028	0.027	NS	NS	0.069	0.019
Cage wash 120 - 144	0.022	0.014	NS	NS	NS	NS	0.031	0.006
Cage wash 144 - 168	0.025	0.019	NS	NS	NS	NS	0.021	0.009
Expired air 0 - 4	0.024	0.023	0.039	0.038	0.054	0.055	0.060	0.066
Expired air 4 - 8	0.000	0.000	0.024	0.018	0.027	0.000	0.004	0.006
Expired air 8 - 12	0.000	0.000	0.010	0.005	0.004	0.000	0.000	0.000
Expired air 12 - 24	0.000	0.000	0.002	0.004	0.000	0.000	0.000	0.000
Expired air 0 - 24h	**0.024**	**0.023**	**0.075**	**0.064**	**0.085**	**0.055**	**0.064**	**0.072**
Expired air 24 - 36	NS	NS	0.000	0.000	NS	NS	NS	NS
Expired air 36 - 48	NS	NS	0.000	0.000	NS	NS	NS	NS
Expired air 48 - 72	NS	NS	0.000	0.000	NS	NS	NS	NS
Faeces 0 - 4	N/A	N/A	NS	NS	NS	NS	NS	NS
Faeces 4 - 8	N/A	N/A	NS	NS	NS	NS	NS	NS
Faeces 8 - 12	1.193	0.502	NS	NS	24.61	17.44	NS	NS
Faeces 12 - 24	1.035	0.631	45.84	40.68	15.58	18.25	38.63	48.27
Faeces 24 - 36	0.530	0.145	8.304	10.94	7.404	8.613	9.788	8.524
Faeces 36 - 48	0.258	0.388	3.126	2.455	1.046	1.498	2.385	1.945
Faeces 0 - 48h	**3.016**	**1.666**	**57.270**	**54.075**	**48.640**	**45.801**	**50.803**	**58.739**
Subtotal Urine/Expired air/faeces 0 - 48h	**87.802**	**84.603**	**85.489**	**83.931**	**82.557**	**79.718**	**84.72**	**80.561**
Faeces 48 - 72	0.191	0.178	1.409	2.158	1.007	0.925	1.612	1.086
Faeces 72 - 96	0.158	0.047	0.121	0.235	NS	NS	0.549	0.505
Faeces 96 - 120	0.073	0.020	0.040	0.059	NS	NS	0.081	0.029
Faeces 120 - 144	0.023	0.000	NS	NS	NS	NS	0.165	0.006
Faeces 144 - 168	0.029	0.000	NS	NS	NS	NS	0.058	0.009

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
N/A not applicable
NS no sample

C. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Radioactivity concentrations in tissues were very low at all times. Radioactive residues were highest in bone. Highest remaining concentrations were detected in carcass.

Table 6.1.1.11-27: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat [redacted] 1992): Radioactivity in tissues (in mean % of applied dose)

Tissue	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	F	M	F	M	F	M	F
Brain	0.003	0.004	0.001	0.002	0.000	0.000	0.000	0.000
Carcass	1.305	1.045	0.576	0.597	0.888	0.771	0.448	0.397
Heart	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000
Kidney	0.009	0.008	0.007	0.005	0.011	0.008	0.004	0.003
Liver	0.030	0.027	0.031	0.027	0.051	0.040	0.017	0.000
Lungs	0.004	0.005	0.002	0.002	0.003	0.003	0.001	0.001
Salivary gland	0.001	0.001	0.000	0.001	0.001	0.001	0.000	0.000
Spleen	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000
Testes/Ovaries	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Uterus	N/A	0.002	N/A	0.001	N/A	0.001	N/A	0.000

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
N/A not applicable

Table 6.1.1.11-28: [^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion in the rat [redacted] 1992): Radioactivity in tissues (in µg equivalents of [^{14}C]-glyphosate/g)

Tissue	Intravenous dose* (30 mg/kg bw)		Oral dose* (30 mg/kg bw)		Oral dose** (30 mg/kg bw)		Oral dose* (1000 mg/kg bw)	
	M	F	M	F	M	F	M	F
Blood	0.050	0.084	0.011	0.000	0.000	0.000	0.000	0.000
Bone	4.195	4.355	2.246	2.562	3.096	2.505	56.32	40.66
Bone marrow	0.255	1.264	0.322	0.545	0.325	0.144	3.080	0.000
Brain	0.118	0.120	0.056	0.056	0.019	0.000	0.000	0.000
Abdominal fat	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
Carcass	0.423	0.335	0.197	0.214	0.339	0.284	5.628	4.476
Heart	0.051	0.025	0.051	0.045	0.000	0.000	0.000	0.000
Kidney	0.304	0.298	0.278	0.205	0.515	0.317	5.170	3.986
Liver	0.241	0.222	0.251	0.254	0.615	0.425	6.144	0.000
Lungs	0.264	0.279	0.124	0.126	0.183	0.173	2.904	1.216
Muscle	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Plasma	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
Salivary gland	0.082	0.068	0.053	0.079	0.084	0.100	0.000	0.000
Spleen	0.117	0.117	0.140	0.091	0.164	0.153	0.000	0.000
Testes/Ovaries	0.000	0.034	0.000	0.068	0.000	0.028	0.000	0.000
Uterus	N/A	0.248	N/A	0.143	N/A	0.239	N/A	0.000

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 consecutive days followed ~24 h later by [^{14}C]-glyphosate)
N/A not applicable

D. URINE AND FAECAL METABOLITES

The profiles of radioactivity for each analytical method were similar for all samples, with no apparent differences between sample type, collection time or sex. Likewise, no differences were observed between dose route, level or frequency.

HPLC analysis
Only one major region of radioactivity was detected (90 to 100 % of total radioactivity) in pooled samples. This corresponded to the retention time for non-radiolabelled and [^{14}C]-glyphosate standards. A region of radioactivity corresponding to AMPA standard was not detected. However, some peak tailing from the major region was observed in this area which may have prevented the detection of very low levels of radioactivity (ca. <5 %).The remainder of the radioactivity was located in diffuse areas.

TLC solvent System 1
One major region of radioactivity (90 - 100 % of total radioactivity in pooled sample) was detected. This co-chromatographed with non-labelled glyphosate reference standard. Up to three minor regions of radioactivity were observed (Region 001, ca. 0 - 8 %, Retention factor (R_f) ca. 0.00; Region 002, ca. 0 - 5 %, R_f ca 0.50; Region 004, ca. 0 - 6 %, R_f ca 0.85). Region 001 was detected at very low levels (<1 %) in most samples. However, in samples containing low amounts of radioactivity, Region 001 was detected at more substantial levels (up to 8 %) and is probably due to non-specific binding of radioactivity at the origin of the TLC plate. Regions 004 (which co-chromatographed with AMPA reference standard) and 002 were either not detected or were only present at low levels (<2 %) in the majority of samples. The remaining radioactivity was located in diffuse areas.

TLC solvent System 2
One major region of radioactivity (Region 002, ca. 81 - 100 % of total radioactivity in pooled sample, R_f ca. 0.20 to 0.30) was detected. This co-chromatographed with non-labelled glyphosate reference standard. Up to two minor regions of radioactivity were also observed (Region 001, ca. 0 - 18 %, R_f ca. 0.00; Region 003, ca. 0 - 3 %, R_f ca. 0.30 - 0.40). Region 001 was detected at very low levels (<1 %) in most samples. However, in samples containing low amounts of radioactivity, Region 001 was detected at more substantial levels (up to 18 %) and is probably due to non-specific binding of radioactivity at the origin of the TLC plate. Region 003 (which co-chromatographed with AMPA reference standard) was either not detected or was only present at low levels (<2 %) in the majority of samples. The remaining radioactivity was located in diffuse areas.

Identification of glyphosate isolated from urine and faeces extracts
The FT-IR spectra obtained, when the major region of radioactivity isolated from both pooled urine and pooled faecal extracts was analysed, showed virtually identical characteristics to the spectra produced when non-labelled and [^{14}C]-glyphosate standards were analysed.

III. CONCLUSIONS

After a single oral dose of 30 mg/kg bw of [^{14}C]-glyphosate, ca. 34 - 36 % were absorbed. The absorption profile did not change on repeated daily administration of 30 mg/kg bw or at a single higher dose level of 1000 mg/kg bw. Most of the dose was voided in faeces reflecting the fraction not absorbed. After intravenous administration, renal excretion was rapid and predominated. Tissue residues were low, higher concentrations in bone were noted. Concentrations in bones increased with dose but not with frequency of dosing. The pattern of absorption, distribution and excretion of [^{14}C]-glyphosate appeared to be independent of sex, and no radiolabelled metabolites were identified in urine or faecal samples.

Assessment and conclusion by applicant:

A single intravenous or oral dose of [^{14}C]-glyphosate at 30 mg/kg bw, a single oral dose of 1000 mg/kg bw, or multiple doses (14 daily oral doses of glyphosate followed by a single oral dose of [^{14}C]-glyphosate on Day 15) at 30 mg/kg bw were administered to Sprague-Dawley rats.

Excretion:
Seven days after single oral low dose administration (30 mg/kg bw) 57 - 59 % and 29 - 31 % of the dose was excreted in faeces and urine (excluding cage wash), respectively. After single oral administration of high dose (1000 mg/kg bw) 53 - 60 % and 23 - 31 % of the dose was excreted in faeces and urine (excluding cage wash), respectively. After multiple doses (14 daily oral doses of glyphosate (30 mg/kg bw/d) followed by a single oral dose (30 mg/kg bw) of [^{14}C]-glyphosate on Day 15), the excretion was 47 - 50 % in faeces and 34 - 35 % in urine (excluding cage wash). After single intravenous dosing at 30 mg/kg bw, 1.5 - 3.4 % and 84 - 86 % of the dose was excreted in faeces and urine (excluding cage wash), respectively.

Absorption and Distribution:
Considering cage wash to calculate the total urinary excretion 92.9 and 97.4 % of the applied radioactivity was excreted in urine by males and females upon intravenous application, respectively. Comparison of mean urinary excretion of radioactivity following single intravenous and single oral administration (30 mg/kg bw) resulted in an oral absorption rate of about 35 % in males and females, respectively. Similar absorption was estimated for multiple and high dose oral administration, indicated by similar proportion of faecal elimination after single low/high and multiple dosing. A high faecal elimination after oral dosing suggests that the rat has limited capacity for absorption of glyphosate since only a small proportion was eliminated in faeces after intravenous dosing. Tissue residues were low; however higher tissue concentrations for bone was noted.

Metabolites:
Chromatographic evaluation of rat urine and faeces indicated that no radiolabelled metabolites of [^{14}C]-glyphosate were detected. The identity of the radioactive component as glyphosate was confirmed by spectrometric method; the same single component was observed after single intravenous and oral doses at 30 and 1000 mg/kg bw and after multiple oral doses at 30 mg/kg bw/d, suggesting there was no change in the pharmacokinetics of glyphosate with time, dose or dose route. Only low levels (<2 %) of radioactivity co-chromatographed with the AMPA reference standard.

Conclusion:
After oral administration of [^{14}C]-glyphosate to rats, there were no differences in the absorption and disposition of radioactivity between 30 and 1000 mg/kg bw doses or after multiple daily oral doses of 30 mg/kg bw/d. Faecal excretion was the major route of elimination (probably unabsorbed compound). Approximately 35 % was absorbed from the gut and subsequently eliminated in urine. Absorption was similar in both sexes. Absorption and excretion appeared to be dose level (30 and 1000 mg/kg bw) and dose frequency (15 daily doses) independent. About 46 - 59 % of the orally administered dose(s) are excreted in faeces and 29 - 35 % are excreted in urine. Total tissue residues (carcass plus whole tissues) after 7 days was <1 % of the administered dose after single or multiple dosing. Radioactive residues showed a high affinity for bone. Sex differences in the rat were not apparent. No radiolabelled metabolites were identified in urine or faecal samples. Only low levels (<2 %) of radioactivity co-chromatographed with the AMPA reference standard. After administration by either dose route, dose level or after multiple dosing there were no apparent sex-linked differences in the absorption, distribution, metabolism and excretion of [^{14}C]-glyphosate. There was no indication for bioaccumulation of glyphosate after single or multiple oral dosing. No metabolites of [^{14}C]-glyphosate were detected.

Investigation of pharmacokinetics in plasma was not in scope of this study.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Most of the compound is excreted within 4 hours post dosing when administered intravenously and within 24 hours after oral dosing. Renal excretion is dominant after intravenous administration. As stated previously absorption is low independent of dose or frequency. Distribution of the compound is wide and residues low. The potential for bioaccumulation is low. Glyphosate is mainly excreted unmetabolized.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.12. Study 12

Data point	CA 5.1.1/013
Report author	[redacted]
Report year	1990
Report title	Identification of metabolites of glyphosate in rats.
Report No	1087
Document No	Not reported
Guidelines followed in study	None reported
Previous evaluation	Not accepted in RAR (2015)

GLP/Officially recognised testing facilities	No, not conducted under GLP
Acceptability/Reliability	**Conclusion GRG:** Category 4b **Conclusion AGG:** The study is considered to be unacceptable due to the low number of animals.

Short description of study design and observations	Two Wistar rats per dose received a single dose by gavage/diet? (not clearly reported) with glyphosate technical labelled with tritium to give a radioactivity of 1.11 x 10^7 Bq per dose. Urine and faeces was collected separately. The animals were sacrificed after 4, 8, 24 and 48 h and 3rd and 7th days after feeding and urine, faeces and blood were collected. At necropsy kidney, liver and skeletal muscles were removed And examined by gel chromatography and (at least some fractions) by HPLC for the occurrence of glyphosate and/or its metabolites.
Short description of results	Some of the fractions showed presence of glyphosate. However, none of the fractions showed the presence of glyphosate-related compounds.
Reasons for why the study is not considered relevant/reliable or not considered as key study	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by applicant:
The study was not submitted by the applicants as they do not have access to the study.

It is noted that in the study only 2 animals were included and therefore the number of animals is too low and would be considered as unacceptable. In addition, the following short comings were noted during the previous evaluation, no information on the test material is available (batch, purity). Also the administration method is unclear as on the one hand gastric lavage (gavage?) was mentioned but it also reports dietary administration. In addition, the description of animals and animal handling was very poor. Overall, it is concluded that due to serious limitations of the study the lack of submission of this study is not considered critical for the renewal.

Assessment and conclusion by RMS:
In the RAR (2015) this study was considered not acceptable due to the serious reporting deficiencies. Despite the limitations noted by the applicant it is also noted that the results are barely reported and it is not really clear what the study author means by them. It is agreed with the conclusion in the RAR and by the applicant. This study is superseded by more recent data that is considered acceptable.

B.6.1.1.13. Study 13

Data point	CA 5.1.1/014
Report author	[redacted]
Report year	1988
Report title	The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration
Report No	[redacted]7215
Document No	Not reported
Guidelines followed in study	None reported

Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes (without certificate). However, when the study was performed, GLP was not compulsory.
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

Executive summary

The absorption, distribution, metabolism and excretion of oral and intravenously administered [^{14}C]-glyphosate have been investigated in male and female Sprague Dawley rats after a single intravenous and oral dose (10 mg/kg bw), a single oral dose (1000 mg/kg bw) and after 15 daily oral doses (10 mg/kg bw/day). Samples were collected for a period of up to 7 days after (last) administration. At sacrifice excreta, tissues, organs and residual carcasses were analysed for radioactivity. Radioactive tissue residues were determined after at least 90 % of the oral and intravenous dose, respectively had been excreted.

The radioactivity in expired gases trapped for 24 h after single intravenous dosing at 10 mg/kg bw was <0.2 % of the applied dose.

Seven days after intravenous dosing at 10 mg/kg bw, 74.5 - 79.0 % of the dose was excreted in the urine (excluding cage wash) and 4.65 - 8.3 % in faeces; less than 0.1 % of the dose was found in the organs and approximately 1 % of the dose in the residual carcass. The total recovery in this experiment was 86 % for males and 85 % of the applied dose for females.

Faeces was the major elimination route after oral dosing at 10 mg/kg bw (62.4 and 69.4 % of the applied dose for males and females, respectively) and at 1000 mg/kg bw (68.9 and 69.4 % of the applied dose for males and females, respectively); urine accounted for 28.6 %/17.8 % for males and 22.5 %/14.3 % for females of the low/high dose, respectively. Less than 0.05 % of the applied dose appeared in the organs following oral dosing and less than 0.5 % remained in the residual carcass. Multiple dosing at 10 mg/kg bw/day had no significant effect on the routes of excretion of [^{14}C]-glyphosate nor on the percent of dose remaining in the organs, tissues and residual carcass at sacrifice.

Radiochemical analysis of individual tissues demonstrated that bone contained the highest relative concentration of [^{14}C]-glyphosate equivalents (0.3 - 31 ppm). It was estimated that only 0.2 - 0.6 % of the applied dose was associated with this site after oral dosing and ca. 1 % following intravenous dosing. The remaining tissues contained between 0.0003 and 11 ppm of glyphosate equivalents. For the bone and some highly perfused tissues, the males contained statistically higher levels than the females.

The half-life of the alpha elimination phase varied between 2.11 and 7.52 h and the beta phase was 69 to 337 h. The half-life of the high dose males was found to be significantly longer than the low dose males. Multiple dosing at the low dose had no significant effect on the whole body elimination.

Plots of blood concentration versus time were used to calculate the area under the curve and estimate the oral absorption of glyphosate. In this manner the percent absorption was found to be 30.3 % for males and 35.4 % for females. This compared favourably with the percent absorption of glyphosate calculated from the oral and intravenous urine data (36.2 % for males and 30.2 % for females).

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification: Glyphosate (N-(phosphonomethyl)glycine)

Description:	Not reported
Lot/Batch #:	Not reported
Purity:	99.8 %
Stability of test compound:	Not reported

2. Radiolabelled test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	Not reported
Radiochemical purity:	>99 %
Specific activity:	5.3 x 10^4 dpm/µg (Groups 1 - 3, 5 - 7) 5.3 x 10^2 dpm/µg (Group 4, high dose group)
Stability of test compound:	Not reported

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	[redacted]
Age:	7 - 14 weeks at the time of dosing
Sex:	Males and females
Weight at dosing:	148 - 336 g
Acclimation period:	At least 7 days
Diet/Food:	Purina Rat Chow pellets from Ralston Purina (St. Louis, MO), *ad libitum.* Groups 2 - 7: fasted overnight prior to dosing with radioactive glyphosate
Water:	Tap water, *ad libitum*
Housing:	Group 1: Roth-type metabolism units in order to measure expired gases. The cage body of the unit was 17 cm in diameter. An inverted glass funnel was suspended above the faecal collector to deflect urine. The feeding tube was separated from the cage unit by a piece of ½ inch screen. A glass wool filter was used to remove particulates from incoming air. The airflow was maintained at 24 to 30 L per hour. Groups 2 - 7: stainless steel suspension metabolism cages with a mesh screen for separating urine from faeces.
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment

Group	Rats per	Route of	Frequency of dosing	Dose level (mg/kg bw)	Mean dose in	Investigations	Sampling period

	sex	**dosing***			**DPM**		
1	3	Oral	Single	10	1.15×10^8	Expired gases, urine and faeces	24 h after dosing
2	3	Oral	Single	10	1.11×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d
3	5	i.v.	Single	10	1.34×10^8	Urine faeces and tissues	6, 12, 24 h after dosing and then at 24 h intervals until necropsy on Day 7
4	5	Oral	Single	1000	1.20×10^8		
5	5	Oral	Single	10	8.99×10^7		
6	5	Oral	Multiple**	10	1.48×10^8		
7	3	i.v.	Single	10	1.34×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d

* oral gavage or IV injection to lateral tail vein
** 14 consecutive daily doses of glyphosate followed by radiolabelled glyphosate
DPM disintegrations per minute

Animal assignment and treatment: Intravenous application (Groups 3 and 7)

Depending on the group assigned three or five rats per sex received intravenous applications of 10 mg [^{14}C]-labelled glyphosate/kg bw into the lateral tail vein. The dose was applied in a volume of 0.19 to 0.28 mL and animals were housed in stainless steel metabolism cages. From the five rats per sex of Group 3 urine and faeces were sampled 6, 12, and 24 h after dosing and then at 24 h intervals until necropsy on Day 7. At necropsy following tissues/organs were taken or sampled for radioactivity measurements: Bone, bone marrow, brain, gastrointestinal tract, fat (abdominal; testicular/ovarian), eye, gonads, heart, kidney, liver, lungs, muscle (abdominal/shoulder), nasal mucosa, plasma, spleen, tail, thyroid, uterus, whole blood, red blood cells and residual carcass.

From the three rats per sex of Group 7 blood was collected at 0.25, 0.50, l, 2, 4, 6, 8, 12, 24, 48, 72, 120 h after treatment. At sacrifice, 168 h after dosing, larger blood samples (approximately 5 - 10 mL) were collected from the posterior vena cava of all animals. No urine or faeces were collected from animals of Group 7.

Animal assignment and treatment: Oral application (Groups 1, 2 and 4 - 6)

Depending on the group assigned three or five fasted rats per sex were treated with the prepared testing solutions per gavage. Rats receiving repeated applications for 14 consecutive days (Group 6) received daily doses of glyphosate followed by radiolabelled glyphosate per gavage.

Following treatment with 10 mg [^{14}C]-labelled glyphosate/kg bw, the rats of Group 1 were housed in Roth metabolism cages allowing collection of expired air for 24 h. At 6, 12 and 24 h after dosing, urine, faeces and expired gases were collected. Cage washes were taken after the final collection period.

Following gavage application of 10 mg [^{14}C]-labelled glyphosate/kg bw blood was collected from the three rats per sex of Group 2 at 0.25, 0.50, l, 2, 4, 6, 8, 12, 24, 48, 72, 120 h after dosing. At sacrifice, 168 h after dosing, larger blood samples (approximately 5 - 10 mL) were collected from the posterior vena cava of all animals. No urine or faeces were collected from animals of Group 2.

Urine and faeces from rats of the dose Groups 4 to 6 were sampled 6, 12, and 24 h after dosing and then at 24 h intervals until necropsy on Day 7. At necropsy following tissues/organs were taken or sampled for radioactivity measurements: Bone, bone marrow, brain, gastrointestinal tract, fat (abdominal; testicular/ovarian), eye, gonads, heart, kidney, liver, lungs, muscle (abdominal/shoulder), nasal mucosa, plasma, spleen, tail, thyroid, uterus, whole Blood, red blood cells and residual carcass.

Dosing Formulation Analysis

The test material for this study was a mixture of unlabelled and [^{14}C]-labelled glyphosate. The dosing solutions were prepared by dissolving the test material in sterile saline. The disintegration per minute per gram of the dose

solution was determined by diluting weighed aliquots with saline and then counting weighed amounts of these diluted solutions.

Measurement of radioactivity

Urine was collected and kept chilled by a circulating water bath. Fresh urine samples were prepared for liquid scintillation counting (LSC) by adding approximately 0.03 - 0.21 mL of sample to 15 mL Instagel® prior to counting. 1 - 2 mL of water or 0.5 N ammonium bicarbonate were added to these samples before counting to prevent glyphosate from precipitating out of the counting solution. The faecal samples were combined with approximately an equal volume of distilled water, homogenized and weighed. Portions (0.05 - 0.40 g) of the homogenized faecal material were transferred to combustion cones lined with a Combustopad, capped with a Combustopad and combusted in a Packard Tricarb B306 Sample Oxidizer using a 1 - 2 minute burn cycle. Samples were run in duplicate. A [^{14}C]-calibration standard and an instrument memory check were run after every twenty experimental samples in order to monitor instrument performance. Whole blood, plasma and blood cells were pipetted onto Combustopads, weighed and then capped with another pad and combusted in the Packard sample oxidizer. Tissues collected at necropsy were combusted or solubilized and duplicate samples processed for LSC. Individual carcasses were frozen, freeze-fractured, lyophilized and ground in a blender. Five aliquots of the resulting powder were combusted for LSC.

Liquid Scintillation Counting

All samples were counted with a Mark III Liquid Scintillation Counter (Model 6882, TM Analytic). Counting efficiencies were determined by external standardization and disintegration per minute by the Mark III DPM calculation accessory using a set of Amersham quench standards. Aqueous samples prepared in Instagel® were counted with efficiencies of 77 - 87 %. Solubilized samples prepared in Dimilume® were counted with efficiencies of 84 - 88 %, while combusted samples had efficiencies of 67 - 78 %.

Data Processing and Statistical Procedures

The liquid scintillation counter data were processed by a computer program written by the data processing group at the Environmental Health Laboratory. In these programs each sample is assigned a unique identification that consists of the EHL study number, a treatment group number, excreta or tissue code, a time interval and a replicate number. The data were assembled, stored and processed by the computer system. The standard error of the mean (SEM) and the Student's t-test were performed to determine the significance of differences between sexes and dose levels.

Kinetic Analysis and Measurement of Absorption

The urine and faecal elimination data were analysed using the "sigma-minus" plotting method. This method is a semi-logarithmic plot of the differences between the percent of dose recovered and the cumulative percent eliminated (urine plus faeces) on the logarithmic scale against time on the linear scale. It is used to characterise the kinetics of whole body elimination. The blood data for Groups 2 and 7 were processed by a computer program written in the Statistical Analysis System (SAS) which utilizes the trapezoidal rule. Straight line segments were drawn between adjacent experimental points of the graph of blood concentration versus time, from time 0 to time t. The area under this curve was obtained by summing the areas of all the trapezoids formed. The extrapolated area under the curve from time t to infinity was also calculated and added to the previous area. The area under the curve (AUC) was the sum of these two areas. The first method of determination of oral absorption was by comparison of AUCs (AUC_{oral} /AUC_{iv} multiplied by $Dose_{iv}/Dose_{oral}$). The second method of determination of oral absorption consisted of division of the extent of urinary excretion of radiolabel following oral administration by the extent of urinary excretion of radiolabel following intravenous administration (% of dose in urine (oral) x 100 divided by % of dose in urine (i.v.)).

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

Single bolus intravenous dose at 10 mg/kg bw (Group 3):
79.0 and 74.5 % of the applied dose was excreted in the urine of males and females, respectively. 4.65 and 8.3 % of the applied dose was excreted in the faeces of males and females, respectively. Less than 0.1 % of the dose

was found in the organs taken at necropsy with approximately one percent of the dose remaining in the residual carcass.

Single oral dose at 10 mg/kg bw (Group 5):
62.4 and 69.4 % of the dose was excreted in faeces of males and females, respectively. 28.6 and 22.5 % in the urine of males and females, respectively. Less than 0.05 % of the dose appeared in the organs and less than 0.5 % remained in the residual carcass.

Single oral dose at 1000 mg/kg bw (Group 4):
68.9 and 69.4 % of the applied dose was excreted in faeces of males and females, respectively. 17.8 and 14.3 % of the applied dose was excreted in the urine of males and females, respectively. Less than 0.05 % of the dose appeared in the organs and less than 0.5 % remained in the residual carcass.

Multiple oral dose at 10 mg/kg bw/day (Group 6):
Repeated dosing at 10 mg/kg bw/day had no significant effect on the routes of excretion of [^{14}C]-glyphosate compared to the single dose application. 61.0 and 70.9 % of the applied dose was excreted in faeces of males and females, respectively and 30.9 and 23.1 % in the urine of males and females, respectively.

Table 6.1.1.13-29: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration [redacted] 1988): Mean (±SEM) recovery of radioactivity (% of applied dose) following administration of [^{14}C]-glyphosate to the rat

Sample		Group 1 Oral dose* 10 mg/kg bw		Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/d	
		M	F	M	F	M	F	M	F	M	F
		0 - 24h#		*0 - 168h*							
Urine		12.2	11.1	79.0	74.5	17.8	14.3	28.6	22.5	30.9	23.1
	±	3.0	2.6	5.9	3.3	2.2	1.3	1.8	3.0	4.3	4.3
Faeces		68.6	70.4	4.65	8.30	68.9	69.4	62.4	69.4	61.0	70.9
	±	5.9	5.6	0.93	1.4	2.1	3.0	2.8	3.1	3.5	5.6
CO2		0.137	0.154	Not assessed, as <0.2 % of the dose found in group 1							
	±	0.028	0.030								
Cage wash		0.539	0.526	0.890	1.30	3.86	8.00	1.30	1.96	0.820	1.96
	±	0.21	0.17	0.28	0.29	0.46	1.3[b]	0.23	0.24	0.18	0.79
Organs/Tissues		Not assessed		0.094	0.052[b]	0.036	0.027	0.046	0.0194[b]	0.047	0.031
	±			0.011	0.005	0.003	0.004	0.003	0.0022	0.0044	0.006
Tail				0.172	0.161	Not assessed as oral dosing					
	±			0.043	0.032						
Residual carcass				1.18	1.04	0.248	0.208	0.395	0.286[c]	0.497	0.315
	±			0.096	0.040	0.046	0.024	0.009	0.032	0.064	0.067
Gastrointestinal contents				0.039	0.039	0.026	0.043	0.023	0.015	0.014	0.010
	±			0.010	0.015	0.008	0.028	0.004	0.002	0.003	0.005
Total tissue* **				**1.485**	**1.292**	**0.31**	**0.28**	**0.46**	**0.32**	**0.56**	**0.36**
Total recovery		**81.4**	**82.8**	**86.0**	**85.3**	**90.9**	**92.1**	**92.8**	**94.2**	**93.3**	**96.3**
	±	**8.8**	**4.8**	**5.6**	**3.8**	**1.4**	**1.2**	**0.94**	**0.78**	**1.4**	**1.9**

Sampling period
* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
*** Carcass plus whole tissues/gastrointestinal contents
SEM = standard error of the mean
(b)significantly different from males by Student's t-test, p <0.01 or (c) p <0.05

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Bone contained the highest relative concentration of [^{14}C]-glyphosate equivalents (0.3 - 31 ppm) corresponding to approximately 0.2 - 0.6 % of the applied dose after oral dosing and approximately 1 % after intravenous dosing. The remaining tissues contained between 0.0003 and 11 ppm of glyphosate equivalents. For the bone and some highly perfused tissues, the males contained statistically higher levels than the females. Repeated dose administration had no significant effect on the percent of dose remaining in the organs, tissues and residual carcass.

Table 6.1.1.13-30: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration [redacted] 1988): Mean radioactivity in tissues and organs (ppm) following administration of [^{14}C]-glyphosate to the rat

Tissue	Group 3 i.v. dose* 10 mg/kg bw		Group 4 Oral dose* 1000 mg/kg bw		Group 5 Oral dose* 10 mg/kg bw		Group 6 Oral dose** 10 mg/kg bw/day	
	M	F	M	F	M	F	M	F
Whole Blood	0.019	0.010[b]	0.328	0.166[b]	0.005	0.003[b]	0.005	0.003[b]
Blood Plasma	0.003	0.003	0.129	0.127	0.002	0.001[c]	0.002	0.002
Red Cells	0.032	0.014[b]	0.517	0.275[b]	0.009	0.004[b]	0.008	0.005[c]
Liver	0.104	0.050[b]	1.91	1.37	0.030	0.014[b]	0.041	0.026
Eye	0.016	0.010	0.655	0.590	0.002	0.0003	0.004	0.003
Brain	0.041	0.036	0.750	0.556	0.007	0.006	0.014	0.011
Kidney	0.106	0.071[c]	1.94	1.35[c]	0.022	0.013[c]	0.033	0.020
Spleen	0.044	0.032[c]	2.61	2.98	0.012	0.007	0.016	0.013
Lungs	0.103	0.079	1.54	1.13	0.015	0.012	0.021	0.017
Heart	0.026	0.017[c]	0.590	0.518	0.006	0.004[c]	0.008	0.006
Thyroid	0.022	0.023	1.50	1.24	0.001	0.0004	0.007	0.010
Testes/Ovaries	0.018	0.022	0.363	0.572	0.003	0.003	0.005	0.008
Uterus	-	0.038	-	0.618	-	0.005	-	0.019
Nasal mucosa	0.074	0.040	1.71	1.79	0.005	0.023	0.032	0.013
Stomach	0.024	0.018	2.38	2.36	0.008	0.004[b]	0.038	0.024
Small Intestine	0.026	0.016[c]	1.90	1.55	0.022	0.018	0.044	0.026
Colon	0.035	0.018[c]	11.0	9.20	0.034	0.016[b]	0.043	0.030
Bone	1.48	1.59	30.6	19.7[c]	0.552	0.313[b]	0.748	0.462[c]
Bone marrow	0.069	0.030[c]	4.10	12.50	0.029	0.007	0.025	0.023
Abdominal muscle	0.008	0.006	0.262	0.214	0.002	0.002[b]	0.003	0.002
Shoulder muscle	0.011	0.033	0.419	0.423	0.004	0.007	0.008	0.006
Abdominal fat	0.005	0.004[c]	0.418	0.457	0.004	0.003	0.006	0.006
Testicular/ovarian fat	0.008	0.005	0.442	0.405	0.005	0.003	0.007	0.006
Tail	0.699	0.611	NS	NS	NS	NS	NS	NS
Residual carcass	0.344	0.337	8.27	7.74	0.106	0.087	0.157	0.101
Total								

* Single dose

** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate

[(b)]significantly different from males by Student's t-test, $p < 0.01$ or [(c)] $p < 0.05$

All values rounded to 3 decimal places.

C. RATES OF ELIMINATION OF RADIOACTIVITY

The urine and faecal data of Groups 3 - 6 were used to estimate the kinetics of whole body elimination. Males and females of Groups 3 - 6 had alpha half-lives of 2.1 - 7.5 and 5.0 - 6.4 h, respectively. The beta half-lives ranged from 69.0 - 181 h for males and 79.9 - 337 h for females. Mean half-life are presented in the table below. The half-life of the high dose males was found to be significantly longer than the low dose males. Pre-treatment with multiple low doses had no significant effect on whole body elimination.

Table 6.1.1.13-31: The Metabolism of Glyphosate in Sprague Dawley Rats - Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites Following Intravenous and Oral Administration [redacted] 1988): Mean half-life of whole body elimination of radioactivity

PK parameter	Group 2		Group 3		Group 4		Group 5		Group 6		Group 7	
	Oral dose* 10 mg/kg bw		i.v. dose* 10 mg/kg bw		Oral dose* 1000 mg/kg bw		Oral dose* 10 mg/kg bw		Oral dose** 10 mg/kg bw/day		i.v. dose* 10 mg/kg bw	
	M	F	M	F	M	F	M	F	M	F	M	F
Alpha rate constant [h^{-1} x 10^{-2}]	-	-	35.0	15.9	13.9	12.6	12.3	13.6	9.60	11.6	-	-
Alpha half-life [h]	-	-	2.11	5.00	5.26	6.44	5.87	6.22	7.52	6.14	-	-
Beta rate constant [h^{-1} x 10^{-3}]	-	-	10.9	9.54	4.43	2.86	8.88	7.15	9.41	5.78	-	-
Beta half-life [h]	-	-	69.0	79.9	181	337	79.0	106	75.2	146	-	-
AUC [µg min/mL]	245	226	-	-	-	-	-	-	-	-	849	662

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
PK Pharmacokinetics
Data fit to a bi-exponential function

D. DETERMINATION OF ABSORPTION

Whole blood radiochemical concentrations were used to calculate the ppm of glyphosate or its equivalents. Semi-logarithmic plots of ppm glyphosate equivalents versus time were constructed and the AUCs were estimated. Using AUCs for calculation, the oral absorption of glyphosate for the males was 30.4 % and the oral absorption for the females was 35.4 %. The percent absorption of glyphosate calculated from the oral and intravenous urine data was 36.2 % for males and 30.2 % for females.

E. RECOVERY OF RADIOACTIVITY

More than 90 % of the administered radioactivity was recovered within 7 days in the low and the high dose groups (Groups 4 to 6) after single and multiple oral application of the test substance. After single intravenous dosing >85 % of the administered radioactivity was recovered (Group 3) and the total recovery in the low dose experiment terminated after 24 h was about 82 % (Group 1). No total recovery for the kinetic experiments (Groups 2 and 7) was derived.

III. CONCLUSIONS

The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated following a single oral dose at 10 or 1000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

A single intravenous or oral dose of [^{14}C]-glyphosate at 10 mg/kg bw, a single oral dose of 1000 mg/kg bw, or multiple doses (14 daily oral doses of glyphosate followed by a single oral dose of [^{14}C]-glyphosate on Day 15) of 10 mg/kg bw/day were administered to Sprague-Dawley rats.

Excretion
Seven days after single oral administration, about 62 - 69 % of dose(s) were excreted in faeces and 14 - 29 % were excreted in urine. Seven days after single intravenous dosing about 5 - 8 % of the dose were excreted in faeces and 75 - 79 % of the dose in urine.

Absorption

Two methods were used to calculate the oral absorption rate. For both the oral absorption of glyphosate was between 30 - 36 % after single and multiple administration.

Distribution
Total tissue residues (carcass plus whole tissues and gastrointestinal content) 7 days after oral dosing was <0.6 % after single or multiple low or high dosing. A higher affinity for bone was noted. There was no indication for bioaccumulation of glyphosate after single or multiple oral dosing.

Metabolism
The alpha plasma half-life (rate of decline in plasma) ranged between 5.3 - 7.5 h after oral administration irrespective of dose level and number of dosing. The alpha plasma half-life after i.v. application ranged between 2 to 5 h. The beta half-lives (rate of decline due metabolism/elimination processes) ranged from 75.2 - 79 h for males and 106 - 146 h for females after single and repeated oral dosing of 10 mg/kg bw. Beta half-lives after i.v. application of 10 mg/kg bw were compared to the beta half-lives after oral application (69 and 79 h in males and females, respectively). Beta half-lives at the high dose were 181 and 337 h for males and females, respectively. Metabolites of glyphosate were not assessed in this part of the study. Characterisation of the radioactivity is described in Part II of this study.

Conclusion:
Absorption and excretion of radioactivity in rats after oral administration was neither dose nor gender depended. Irrespective of dose and gender faecal excretion was the major route of elimination and reflected probably unabsorbed compound. Only approximately 30 - 36 % of the applied radioactivity was absorbed from the gut and subsequently eliminated in urine. Absorption and excretion of [^{14}C]-glyphosate was also independent from the application frequency (i.e. comparable between single and repeated daily applications for 15 consecutive days). Overall tissue residues were low and no indication for bioaccumulation of glyphosate after single or multiple oral dosing was observed. Highest radioactivity level was detected in bones.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Absorption of the compound is low, distribution wide and excretion fast. Absorption and excretion are not dependent on dose or frequency of dosing. Renal excretion is dominant after intravenous administration.
Tissue residues are low, although in this study slightly higher in males compared to females. The concentration in tissues is dose dependent, but does not show signs of bioaccumulating. The highest radioactivity level was detected in bones.
The half-life of the compound was longer in high dose animals compared to low dose animals.
This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.14. Study 14

Data point	CA 5.1.1/015
Report author	[redacted]
Report year	1988
Report title	The Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, Characterization, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration
Report No	[redacted] 7206
Document No	Not reported
Guidelines followed in study	US-EPA FIFRA 85-1
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially	Yes , however, when the study was performed GLP was not compulsory.

recognised testing facilities	
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

Executive summary

A full range ADME (absorption, distribution, metabolism and excretion) study conducted in Sprague-Dawley rats was documented in two separate study reports. In Part I (please refer to Section CA 5.1.1/014) animal husbandry, dosing of glyphosate (oral and intravenous), excretion, distribution (measurement of residues in tissues and organs at sacrifice) and oral absorption rates was reported. In this part (Part II) of the study report details on the quantitation, CHARACTERISATION, and identification of glyphosate and its metabolites were provided.

[^{14}C]-Glyphosate was administered to male and female Sprague Dawley rats as single intravenous and oral dose at 10 mg/kg bw, as single oral dose at 1000 mg/kg bw and at 15 daily oral doses at 10 mg/kg bw/day. Samples were collected for a period of up to 7 days after (last) administration. At sacrifice excreta, tissues, organs and residual carcasses were analysed for radioactivity. Radioactive tissue residues were determined after at least 90 % of the oral dose had been excreted. Orally administered glyphosate was absorbed to the extent of 30 - 36 %.

[^{14}C]-Glyphosate was the predominant material found in the rat excreta as revealed by two different methods of HPLC analyses, cation exchange HPLC and ion pair HPLC. Glyphosate was isolated from urine (overall recovery of 81.3 %) and from faeces (overall recovery of 99.2 %) and was positively identified by different analytical methods. At least 97.5 % of the urine or faecal extract-contained radioactivity was [^{14}C]-glyphosate, in all of the individual rat excreta samples. The HPLC analyses indicated that glyphosate in the excreta accounted for >98.5 - 99.3 % of the administered dose. There was evidence for formation of 0.2 - 0.4 % aminomethylphosphonic acid (AMPA) from *in vivo* metabolism after oral administration of glyphosate at single or multiple low dose level. The remainder of the excreta-contained radioactivity was due to low-level impurities present in the dosing material or due to materials that formed during storage of the excreta samples by reaction of glyphosate with endogenous excreta components.

I. MATERIALS AND METHODS

A. MATERIALS

1. Non-labelled test material:

Identification: Glyphosate (N-(phosphonomethyl)glycine)

Description: Not reported

Lot/Batch #: Not reported

Purity: 99.9 % (determined by strong anion exchange (SAX) HPLC assay)
99.81 % with [redacted] present as impurity (determined by silylation of the glyphosate with [redacted] and GC analysis on a 3 % DC-200 GC column)

Stability of test compound: Not reported

2. Radiolabelled test material:

Identification: [^{14}C]-Glyphosate

Position of radiolabel: N-(phosphono[^{14}C]methyl)glycine

Lot/Batch #: Not reported

Radiochemical purity: >99 %

Specific activity: 5.3×10^4 dpm/µg (Groups 1 - 3, 5 - 7)
5.3×10^2 dpm/µg (Group 4, high dose group)

Stability of test compound: Not reported

3. Reference substances:

Identification: [^{14}C]-Aminomethylphosphonic acid (AMPA)
Description: Not reported
Lot/Batch #: Not reported
Purity: Not reported
Stability of test compound: Not reported

Identification: [^{14}C]-N-methylaminophosphonic acid (MAMPA)
Description: Not reported
Lot/Batch #: Not reported
Purity: 97.3 % (CX HPLC analysis)
Stability of test compound: Not reported

Identification: N-nitroso-glyphosate
Description: Not reported
Lot/Batch #: Not reported
Purity: 87 % (IPC analysis)
Stability of test compound: Not reported

4. Test animals:

Species: Rat
Strain: Sprague-Dawley (Crl:CD BR)
Source: [redacted]
Age: 7 - 14 weeks at the time of dosing
Sex: Males and females
Weight at dosing: 148 - 336 g
Acclimation period: At least 7 days
Diet/Food: Purina Rat Chow pellets from Ralston Purina (St. Louis, MO), *ad libitum.*
Groups 2 - 7: fasted overnight prior to dosing with radioactive glyphosate
Water: Tap water, *ad libitum*
Housing: Group 1: Roth-type metabolism units in order to measure expired gases. The cage body of the unit was 17 cm in diameter. An inverted glass funnel was suspended above the faecal collector to deflect urine. The feeding tube was separated from the cage unit by a piece of ½ inch screen. A glass wool filter was used to remove particulates from incoming air. The airflow was maintained at 24 to 30 L per hour.
Groups 2 - 7: stainless steel suspension metabolism cages with a mesh screen for separating urine from faeces.
Environmental conditions: Temperature: Not reported
Humidity: Not reported
Air changes: Not reported
12-hour light/dark cycle

B. STUDY DESIGN

In life dates: not reported

Animal assignment and treatment

A full range ADME (absorption, distribution, metabolism and excretion) study conducted in Sprague-Dawley rats was documented in two separate study reports. Details of animal husbandry and treatment, dosing of glyphosate (oral and intravenous), excretion, distribution (measurement of residues in tissues and organs at sacrifice) and oral absorption rates are provided in Section B6.1.1.13). The study design is summarised below.

Group	Rats per sex	Route of dosing*	Frequency of dosing	Dose level (mg/kg bw)	Mean dose in DPM	Investigations	Sampling period
1	3	Oral	Single	10	1.15×10^8	Expired gases, urine and faeces	24 h after dosing
2	3	Oral	Single	10	1.11×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d
3	5	i.v.	Single	10	1.34×10^8	Urine faeces and tissues	6, 12, 24 h after dosing and then at 24 h intervals until necropsy on Day 7
4	5	Oral	Single	1000	1.20×10^8		
5	5	Oral	Single	10	8.99×10^7		
6	5	Oral	Multiple**	10	1.48×10^8		
7	3	i.v.	Single	10	1.34×10^8	Blood at frequent intervals	5 d post dosing and at sacrifice after 7 d

* oral gavage or IV injection to lateral tail vein
** 14 consecutive daily doses of glyphosate followed by radiolabelled glyphosate
DPM disintegrations per minute

Sampling

The identity of the radioactivity was characterised of urine and faeces sampled 6, 12, and 24 h after dosing and then at 24 h intervals until necropsy on Day 7 from rats of Groups 3 - 6.

Pooling of Rat Excreta for Analyses

For each rat in Group 3 (intravenous) and Groups 4 - 6 (oral), aliquots (10 % each) of the urine samples collected sequentially after dosing that contained together >94 % of the total radioactivity excreted during the entire collection period were pooled. Similar pooling of faecal samples was done. This gave pooled urine and pooled faecal samples for each rat of Groups 3 - 6. In addition, aliquots (10 % each) of the pooled urine samples prepared for each of the males in Group 3 were combined, giving a pooled male Group 3 urine sample. In identical fashion, pooled urine samples for females and males of Groups 3 to 6 were prepared.

Sample Preparation

Urine samples were analysed by HPLC without prior treatment.
Faecal samples were extracted with water. The extracts were cleaned up using C_{18} solid phase extraction cartridges, concentrated by rotary evaporation, and re-dissolved in the HPLC buffer prior to analysis. The percent of faecal-contained radioactivity that was extracted (normalized) was 83 - 91 % for Groups 3, 5, and 6. For Group 4, the high dose group, the extractabilities (normalized) were 96 - 98 %. Recoveries for the clean-up and concentration step were typically 96 - 100 %. Extraction of two Group 3 faecal samples with 1 N HC1 resulted in 99.9 %, extractability compared with 84.6 % extractability with just water, but adjustment of the pH of the extract (after concentration and re-dissolution in HPLC mobile phase) to higher values appropriate for HPLC analyses resulted in precipitation of 90 % of the radioactivity. The precipitation of radioactivity was decreased to 50 % by incorporation of EDTA in the solvent. These results suggest that complexation of glyphosate with metal ions (or other endogenous materials that were not proteins, since proteins would not be soluble in 1 N HC1) occurs to a minor extent in faeces and that acid extraction dissolves the complexation agents. To avoid losses of radioactivity due to precipitation as the pH of the acid extract was raised by the HPLC mobile phase and to avoid metal poisoning of the cation exchange column employed for HPLC analyses as much as possible (a problem that occurred even with nonacidic extracts of faeces), water was employed for extractions of faecal samples.

HPLC Analyses of Excreta

Two different HPLC methods, CX HPLC and IPC, were employed for analysis of urine and faecal samples. Fraction collection of the HPLC effluent and liquid scintillation counting of the fractions (HPLC/ LSC) were employed in order to maximize sensitivity of detection of low level radioactive components. Column performance was monitored with radiolabelled standards and a radioactivity flow detector (RAD). CX HPLC on a Bio-Rad Aminex A-9 column was employed to analyse the pooled urine samples and pooled faecal extracts for each rat in Groups 3 - 6 and the pooled urine samples for each sex in Groups 3 - 6. IPC was used to analyse the pooled urine and pooled faecal extracts for each sex in Groups 3 - 6.

Isolation and Identification of Glyphosate from Urine and Faeces

Glyphosate was isolated from a rat urine sample collected 0 - 6 h after dosing by ion exchange chromatography. The isolated glyphosate (overall recovery of 81.3 %) was characterised by HPLC, negative ion FAB mass spectrometry, [^{1}H] and [^{31}P]-NMR, and by GC/MS analysis of the trifluoroacetic anhydride/trifluoroethanol (TFAA/TFE) derivative.
Glyphosate was isolated from an extract of a rat faecal sample collected 6 - 12 h after dosing by ion exchange chromatography. The isolated glyphosate (overall recovery of 99.2 %) was characterised by HPLC, negative ion FAB mass spectrometry, [^{1}H] and [^{31}P]-NMR, and GC/MS analyses of the TFAA/ TFE derivative.

Sample Stability Studies

Stability studies were conducted to determine the stability of (1) a mixture of glyphosate and a small amount of AMPA, a potential metabolite, in urine; of (2) glyphosate in urine and faeces; and of (3) glyphosate-derived metabolites in a urine sample collected from a dosed rat. Stability samples were analysed with CX HPLC and/or IPC. The stability of glyphosate, the test substance, was determined by spiking samples of control rat urine and faeces, with subsequent handling and storage as for the un-spiked samples.

HPLC setup

The HPLC instrumentation employed in this study consisted of various combinations of Waters Model U6K injectors, Model 440 UV detectors employed at 254 nm, a Waters Model 490 programmable multiwavelength detector, Waters Model 6000A HPLC pumps, Waters Model 510 HPLC pumps, Waters Model 680 automated gradient controllers, and a Waters Model 720 systems controller.

The dosing solutions used for the treatment of the rats of Groups 1 and 3 to 6 were analysed for AMPA and glyphosate content at the time of preparation by strong anion exchange (SAX) HPLC. Subsequently, better HPLC methods of analysis were developed; the unused dosing solutions for Groups 1 and 3 - 6 were analysed for several compounds by cation exchange (CX) HPLC and by ion pair HPLC (IPC). These HPLC methods allowed analysis for glyphosate (1), AMPA (2), MAMPA (3), N-formylglyphosate (4), N-acetylglyphosate (5), N-nitrosoglyphosate (6), N-methylglyphosate (7), methylphosphonic acid (8), hydroxymethylphosphonic acid (9), phosphonoformic acid (10), and an unknown compound (11).

Liquid Scintillation Counting

All samples were counted with a Mark III Liquid Scintillation Counter (Model 6881, TM Analytic).

Gas Chromatograph

A Varian Model 3700 gas chromatograph with a flame ionization detector was employed for analysis of the trifluoroacetic anhydride/trifluoroethanol derivatization reactions.

Combustor (Sample Oxidizer)

Samples were combusted using a Packard Tri-Carb sample oxidizer (Model 306).

Mass Spectrometers

Chemical ionization (Cl) and electron impact (El) mass spectra were obtained on a Finnigan 4535 quadrupole mass spectrometer with an INCOS data system. Fast atom bombardment (FAB) mass spectra were recorded on a VG ZAB-HF double-focusing mass spectrometer and processed with a Digital PDP 11/24 data system.

Nuclear Magnetic Resonance Spectrometer

NMR spectra ([^{1}H] and [^{31}P]) were obtained on a Varian XL-300 NMR spectrometer.

Data Processing and Statistical Procedures

In HPLC/LSC analyses of urine samples, the injected dpm value typically was 50,000. For a background count per minute (cpm) of 14, the threshold was ca. 20. Thus, ca. 8 dpm of sample (ca. 0.02 % of 50,000 injected dpm) in one sample vial could be detected. If the dpm for a metabolite were spread among two or more vials, the sensitivity would be less.
In CX HPLC/RAD analyses employing the 0.5 mL RAD cell the detection limits (at 2 times background) were 110 dpm for nonbasic compounds, 144 dpm for N-methylglyphosate, 201 dpm for MAMPA, and 228 dpm for AMPA (optimized MACS peak width parameter = 500).

II. RESULTS AND DISCUSSION

A. ANALYSES OF DOSING SOLUTIONS

The composition of dosing solutions were analysed by three different methods as shown in the table below. In all study groups, administered doses consisted of at least 98.2 % glyphosate. The main impurity in the dosing solution was [redacted]

Table 6.1.1.14-32: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration: HPLC analyses of dosing solutions

Sample	Method	Distribution [%]							HPLC recovery [%]
		Gly	AMPA	MAMPA	N-Ac-gly	N-F-gly	N-NO-gly	Unk.	
Group 1 Oral dose* 10 mg/kg bw	SAX	>99.10	0.10		N/A				97.6
	CX	98.42	0.63	0.26	0.56				99.6
	IPC	98.21	0.89		≤0.04	0.48	≤0.05	≤0.06	96.6
Group 3 IV dose* 10 mg/kg bw	SAX	>98.90	0.28		N/A				101.3
	CX	99.33	0.36	0.00	0.32				99.2
	IPC	99.14	0.39		≤0.02	0.36	≤0.01	0.03	99.2
Group 4 Oral dose* 1000 mg/kg bw	SAX	98.26	0.74		N/A				98.2
	CX	99.00	0.57	0.31	0.13				98.6
	IPC	98.88	0.83		≤0.03	0.14	≤0.02	0.04	99.7
Group 5 Oral dose* 10 mg/kg bw	SAX	>99.40	0.10		N/A				103.9
	CX	99.61	0.17	0.00	0.15				98.1
	IPC	99.41	0.20		≤0.03	0.18	≤0.03	≤0.03	95.0
Group 6 Oral dose** 10 mg/kg bw/day	SAX	>99.30	0.10		N/A				96.1
	CX	99.50	0.19	0.07	0.14				101.9
	IPC	99.36	0.27		≤0.03	0.21	≤0.02	≤0.02	98.0

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
N-Ac N-acetyl
N-F N-formyl
N-NO N-nitroso
Unk. unknown
N/A not analysed
SAX strong anion exchange HPLC
CX cation exchange HPLC
IPC gradient ion pair HPLC

B. HPLC ANALYSES OF EXCRETA

Retention times of the radioactive components assigned as glyphosate and AMPA matched those of authentic synthetic standards both in the CX and IPC-HPLC analyses.

CX HPLC Analyses

All the pooled samples for the individual rats were analysed by CX HPLC. The analysis of dosing solutions, the metabolite levels as percentages of distribution and recovery rates are given in the table below.

Table 6.1.1.14-33: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration: Mean metabolite concentration in % of urine/faeces-contained radioactivity (CX HPLC)

	Sample		Distribution [%]				HPLC recovery of injected dpm [%]
			Gly	AMPA	MAMPA	Non-basic compounds	
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.33*	*0.36*	*ND*	*0.32*	*99.2*
	Urine	M	99.74	0.17	ND	0.08	97.3
		F	99.56	0.23	ND	0.19	96.9
	Faeces	M	98.78	0.66	ND	0.47	94.6
		F	99.05	0.50	ND	0.27	96.3
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*99.00*	*0.57*	*0.31*	*0.13*	*98.6*
	Urine	M	98.12	0.76	0.30	0.69	97.2
		F	97.98	0.78	0.30	0.77	97.2
	Faeces	M	98.86	0.55	0.32	0.17	97.8
		F	98.78	0.61	0.31	0.20	98.5
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.61*	*0.17*	*ND*	*0.15*	*98.1*
	Urine	M	99.33	0.26	ND	0.32	99.0
		F	99.21	0.25	ND	0.42	99.0
	Faeces	M	99.20	0.42	ND	0.25	99.8
		F	99.09	0.52	ND	0.26	97.7
Group 6 Oral dose** 10 mg/kg bw/day	*Dose*		*99.50*	*0.19*	*0.07*	*0.14*	*101.9*
	Urine	M	99.46	0.25	0.01	0.24	98.9
		F	99.14	0.27	0.02	0.44	99.1
	Faeces	M	98.78	0.74	ND	0.35	97.6
		F	98.76	0.74	ND	0.35	98.0

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
ND not detected
CX cation exchange HPLC
dpm disintegrations per minute
Analyses of the dosing solutions in italics

For calculations of the factors for conversion of percent of distribution to percent of dose, the radioactivity in the cage washes for Groups 3 and 5 - 6 (0.82 - 1.96 % of the dose) was assigned to urine and faeces in the same proportion as the radioactivity levels found in the urine and faeces in these groups. In Group 4, the radioactivity levels in the cage wash (4 - 8 % of the dose) were higher than in the other groups and appeared to be associated mainly with faecal contamination of the cage due to some minor diarrhoea in these high dose animals. Therefore, the radioactivity in the cage wash for this group was attributed to the faeces for the calculations.

Metabolite levels as percentages of administered radioactive dose are given in the table below.

Table 6.1.1.14-34: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration: Mean metabolite concentration as % of administered radioactivity (CX HPLC)

	Sample		Administered ^{14}C [%]			
			Gly	**AMPA**	**MAMPA**	**Non-basic compounds**
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.33*	*0.36*	*ND*	*0.32*
	Urine	M	92.44	0.16	ND	0.08
		F	88.08	0.20	ND	0.19
	Faeces	M	5.39	0.04	ND	0.47
		F	9.77	0.05	ND	0.27
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*99.00*	*0.57*	*0.31*	*0.13*
	Urine	M	19.21	0.15	0.06	0.14
		F	15.22	0.12	0.05	0.12
	Faeces	M	79.13	0.44	0.26	0.14
		F	83.01	0.51	0.26	0.17
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.61*	*0.17*	*ND*	*0.15*
	Urine	M	31.01	0.08	ND	0.12
		F	24.16	0.06	ND	0.02
	Faeces	M	67.58	0.29	ND	0.17
		F	74.44	0.39	ND	0.20
Group 6 Oral dose** 10 mg/kg bw/day	*Dose*		*99.50*	*0.19*	*0.07*	*0.14*
	Urine	M	33.21	0.08	ND	0.08
		F	24.25	0.07	ND	0.11
	Faeces	M	65.12	0.49	ND	0.23
		F	74.14	0.56	ND	0.26

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
ND not detected
CX cation exchange HPLC
Analyses of the dosing solutions in italics

IPC analyses of the pooled male and pooled female urine and faecal samples for Groups 3 - 6 expressed as distribution values, as well as the analytical values for the dosing solutions are given in the table below.

Table 6.1.1.14-35: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration: Mean metabolite concentrations in excreta (IPC HPLC) as % radioactivity counted in HPLC effluent

	Sample		Distribution [%]						HPLC recovery of injected dpm [%]
			Gly	**AMPA**	**N-Ac-gly**	**N-F-gly**	**N-NO-gly**	**Unk.**	
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.14*	*0.39*	*≤0.02*	*0.36*	*≤0.01*	*0.03*	*99.2*
	Urine	M	99.49	0.19	≤0.03	0.07	0.06	0.14	99.4
		F	99.18	0.22	0.07	0.07	0.06	0.40	99.9
	Faeces	M	98.57	0.59	0.00	0.00	0.32	0.44	99.8
		F	99.28	0.46	0.00	0.00	0.10	0.15	97.2
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*98.88*	*0.83*	*≤0.03*	*0.14*	*≤0.02*	*0.04*	*99.7*
	Urine	M	97.76	1.25	0.10	0.20	0.09	0.46	99.2
		F	97.71	1.39	≤0.05	0.25	0.09	0.33	100.6
	Faeces	M	98.64	0.82	≤0.03	≤0.04	0.13	0.16	98.6
		F	98.68	0.88	≤0.04	≤0.04	0.11	0.17	98.7
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.41*	*0.20*	*≤0.03*	*0.18*	*≤0.03*	*≤0.03*	*95.0*
	Urine	M	99.05	0.32	≤0.05	0.12	0.11	0.31	105.1
		F	98.65	0.30	≤0.06	0.25	0.11	0.58	99.9
	Faeces	M	98.78	0.56	≤0.06	≤0.10	0.21	0.16	99.0
		F	98.23	0.64	≤0.05	≤0.09	0.22	0.16	98.2
Group 6 Oral dose**	*Dose*		*99.36*	*0.27*	*≤0.03*	*0.21*	*≤0.0.2*	*≤0.02*	*98.0*
	Urine	M	99.24	0.29	≤0.05	0.11	0.08	0.18	100.1

10 mg/kg bw/day	Faeces	F	98.84	0.26	≤0.04	0.12	0.15	0.51	100.3
		M	98.31	0.90	≤0.06	≤0.10	0.24	0.17	99.0
		F	98.27	0.93	≤0.05	≤0.10	0.22	0.23	98.8

*single; **multiple; gly = glyphosate; N-Ac = N-acetyl; N-F = N-formyl; N-NO = n-nitroso; Unk. = unknown; IPC = gradiant ion pair HPLC, dpm = disintegrations per minute
Analyses of the dosing solutions in italics

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
N-Ac N-acetyl
N-F N-formyl
N-NO N-nitroso
Unk. unknown
IPC gradient ion pair HPLC
dpm disintegrations per minute
Analyses of the dosing solutions in italics

N-nitroso-glyphosate was present at 0.06 - 0.15 % of the urine-contained radioactivity and at 0.10 - 0.32 % of the faecal contained radioactivity. N-formyl-glyphosate was detected in urine at 0.07 - 0.25 % of the urinary contained radioactivity but not in faeces. AMPA concentrations in urine ranged from 0.26 - 1.39 % of the urine-contained radioactivity and from 0.56 - 0.93 % in faeces upon oral application.

Yields of the radiolabelled excreta components as percentages of administered radioactivity are given in the table below. The factors for conversion of distribution values to percent of dose values were the same as employed in CX HPLC.

Table 6.1.1.14-36: Metabolism of Glyphosate in Sprague Dawley Rats - Part II. Identification, CHARACTERISATION, and Quantitation of Glyphosate and Its Metabolites After Intravenous and Oral Administration: Mean metabolite concentrations as % of administered radioactivity (IPC HPLC)

	Sample		**Administered ^{14}C [%]**					
			Gly	**AMPA**	**N-Ac-gly**	**N-F-gly**	**N-NO-gly**	**Unk.**
Group 3 i.v. dose* 10 mg/kg bw	*Dose*		*99.14*	*0.39*	*≤0.02*	*0.36*	*≤0.01*	*0.03*
	Urine	M	92.21	0.18	≤0.03	0.06	0.06	0.13
		F	87.74	0.19	0.06	0.06	0.05	0.35
	Faeces	M	5.38	0.03	0.00	0.00	0.02	0.02
		F	9.79	0.05	0.00	0.00	0.01	0.01
Group 4 Oral dose* 1000 mg/kg bw	*Dose*		*98.88*	*0.83*	*≤0.03*	*0.14*	*≤0.02*	*0.04*
	Urine	M	19.14	0.24	0.02	0.04	0.02	0.09
		F	15.17	0.22	≤0.01	0.04	0.01	0.05
	Faeces	M	78.95	0.66	≤0.02	≤0.03	0.10	0.13
		F	82.93	0.74	≤0.03	≤0.03	0.09	0.14
Group 5 Oral dose* 10 mg/kg bw	*Dose*		*99.41*	*0.20*	*≤0.03*	*0.18*	*≤0.03*	*≤0.03*
	Urine	M	30.92	0.10	≤0.02	0.04	0.03	0.10
		F	24.02	0.07	≤0.01	0.06	0.03	0.14
	Faeces	M	67.29	0.38	≤0.04	≤0.07	0.14	0.11
		F	73.79	0.48	≤0.04	≤0.07	0.17	0.12
Group 6 Oral dose** 10 mg/kg bw/day	*Dose*		*99.36*	*0.27*	*≤0.03*	*0.21*	*≤0.0.2*	*≤0.02*
	Urine	M	33.14	0.10	≤0.02	0.04	0.03	0.06
		F	24.18	0.06	≤0.01	0.03	0.04	0.12
	Faeces	M	64.81	0.59	≤0.04	≤0.07	0.16	0.11
		F	73.77	0.70	≤0.04	≤0.08	0.17	0.17

* Single dose
** Multiple dose (non-radiolabelled glyphosate for 14 days followed by [^{14}C]-glyphosate
Gly glyphosate
N-Ac N-acetyl
N-F N-formyl
N-NO N-nitroso
Unk. unknown

IPC gradient ion pair HPLC
Analyses of the dosing solutions in italics

Total yields of N-nitroso-glyphosate in rat excreta ranged from 0.01 - 0.2 % of the administered dose. In the oral dose groups, the yields of N-nitroso-glyphosate were always greater in the faeces than in the urine. Less than 0.1 % N-acetylglyphosate was formed. No detectable amounts of hydroxylmethylphosphonic acid or methylphosphonic acid were found in any of the samples. Slight increases in amounts of AMPA were noted in the excreta of rats of Groups 5 and 6 but not of Groups 3 and 4, in agreement with the results found by CX HPLC.

C. STABILITY ANALYSES

In all of the stability samples, the variation of amounts of glyphosate with time was minor and appeared to be within the range of measurement errors. The recovery ranged from 97.94 % - 99.8 % of the spikes glyphosate.
The excreta stability analyses showed formation of 0.05 - 0.13 % N-nitrosoglyphosate in urine. Faeces spiked with glyphosate (which contained <0.03 % N-nitrosoglyphosate) showed formation of 0.15 % N-nitrosoglyphosate, which can be a result of reaction of glyphosate with nitrite naturally occurring in the urine and faeces. The sample stability studies also showed formation of up to 0.22 - 0.28 % unknown compound, that eluted last in the IPC analyses.

D. CHARACTERISATION OF UNKNOWN

Efforts to characterise the unknown compound indicated that this material was non-basic (it eluted in the void volume upon CX HPLC) and generated glyphosate and perhaps AMPA upon hydrolysis in pH 2.1 phosphate buffer. The latest eluting radioactive component in IPC of rat excreta did not correspond in retention time to any standard on hand. Its structure was not determined.

III. CONCLUSIONS

Metabolism of glyphosate occurs only to a minor extent in Sprague-Dawley rats, regardless of whether the route of administration is intravenous or oral. Traces of AMPA (0.2 - 0.4 %) appeared to form in rats administered single or multiple oral doses of glyphosate at 10 mg/kg bw. Since the amount of AMPA was so small (<1 %), the identification of AMPA was based solely on the HPLC retention times in the two radically different methods of HPLC. The results of the stability studies and the finding of the majority of the N-nitroso glyphosate (total yields of 0.06 - 0.50 %) in the faeces suggest strongly that the observed N-nitroso glyphosate is not a result of metabolism in the rat but rather is a result of chemical reaction of nitrite naturally occurring in the excreta with glyphosate.

The overall results of this study show that orally-administered glyphosate is absorbed to the extent of 30 - 36 %, metabolism of glyphosate in the rat is very minor, and AMPA, the sole metabolite, appears to be formed at <1 % by *in vivo* metabolism of glyphosate in rats dosed orally at 10 mg/kg bw, either in single or multiple doses.

Assessment and conclusion by applicant:

A full range ADME (absorption, distribution, metabolism and excretion) study in Sprague-Dawley rats was documented in two separate study reports. In this study report the results on metabolites were provided.

The predominant radioactive component with at least 97.5 % of the urine or faecal extract-contained radioactivity was [^{14}C]-glyphosate. The HPLC analyses of dosing solutions and excreta samples indicated that glyphosate in the excreta accounted for >98.5 - 99.3 % of the administered dose. Only small amounts (<1 %) of aminomethylphosphonic acid (AMPA) were found in rats administered single or multiple oral doses of [^{14}C]-glyphosate. Amounts of glyphosate and AMPA in excreta were at similar extents when comparing the results of two different HPLC methods (cation exchange and ion pair HPLC).

Total yields of N-nitroso-glyphosate in rat excreta ranged from 0.06 - 0.20 % of the administered dose and less than 0.1 % N-acetylglyphosate was formed. The fact that the majority of the N-nitroso glyphosate (total yields of 0.06 - 0.50 %) was excreted in faeces suggest that N-nitroso glyphosate is not a result of metabolism in the rat but rather is a result of chemical reaction of nitrite naturally occurring in the excreta with glyphosate.

No detectable amounts of hydroxymethylphosphonic acid or methylphosphonic acid were found. The remainder of the radioactivity detected in the excreta was attributed to low-level impurities present in the dosing material or to materials that had formed during storage of the excreta samples.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. Glyphosate is excreted mainly unmetabolized. Less than 1% is excreted as AMPA. The metabolic profile is independent of sex, dose, method of dosing or frequency of dosing. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.1.15. Study 15

Data point	CA 5.1.1/016
Report author	[redacted]
Report year	1973
Report title	CP 67573 residue and metabolism. Part 13: The dynamics of accumulation and depletion of orally ingested N-phosphonomethylglycine-^{14}C
Report No	309
Document No	Not reported
Guidelines followed in study	None reported
GLP/Officially recognised testing facilities	No, not conducted under GLP (not compulsory at time of study conduct)
Previous evaluation	Yes, supplementary in RAR (2015)
Short description of study design and observations	[^{14}C]-Glyphosate (assumed to be manufactured by [redacted] purity not given) was fed to groups of Wistar rats comprising 16 (treated groups) or 12 (controls) male and female animals each for a period of up to 14 days followed by a recovery period of up to 10 days on normal diet. The dietary levels were 1, 10 and 100 ppm. Faeces and urine were analysed daily for radioactivity. Two animals per sex and dose were sacrificed on treatment Days 2, 6, 10 and 14 and on Days 1, 3, 6 and 10 after withdrawal of dosed feed. No control animals were killed at the second day of medication and on the first day of the recovery period since the number of control rats was too small. Following sacrifice, tissues (liver, kidney, heart, spleen, gonads, brain, muscle, adipose tissue and gut) were examined for radioactive residues.
Short description of results	By Day 4 of dosing, combined excretion of [^{14}C]-activity in urine and faeces exceeded 90 % of the cumulative intake. After six days, the overall excretion was approximately equal to the total intake of [^{14}C]-glyphosate and this was confirmed for all dietary levels at the end of the 14-day dosing period. Thus, it was established that the body load had plateaued at a level directly proportional to the administered dose. Upon withdrawal from dosed feed, the excretion of radioactivity dropped significantly but plateaued temporarily after four days. This was assumed to be due to mobilization of previously established body loads. Most tissues reached maximum residue levels during the dosing period in 10 days or less. Highest tissue concentrations were found in kidneys and spleen. However, bone was not investigated in this study. The tissue residues were clearly not bound. As soon as the exogenous supply of [^{14}C]-glyphosate was removed, the residues began to decrease. The urinary excretion was 8.3 - 10.5 % of the daily intake. The occurrence of faecal excretion during a treatment-free recovery interval after a 14-day dosing period was presumably due to biliary elimination.
Reasons for why the study is not considered relevant/reliable or not	**Conclusion GRG:** Supplementary due to reporting deficiencies, Category 2a Dose levels appear too low for meaningful and reliable residue analysis; however, this is the only dietary study on toxicokinetics of glyphosate in rats.

considered as key study	As the report was received at a late stage of the dossier preparation no full summary was prepared. **Conclusion AGG:** The study is considered to be unacceptable due to the low dose levels. In the RAR (2015) this study was considered supplementary because it was the only toxicokinetic experiment in which glyphosate was administered (for two weeks) to rats via the diet and it was discussed that although dose levels are low they might reflect actual residue concentrations. New studies are now available which supersede this study.

B.6.1.1.16. Study 16

Data point	CA 5.1.1/017
Report author	[redacted]
Report year	1973
Report title	CP 67573 residue and metabolism. Part 9: The gross distribution of n-phosphonomethylglycine-^{14}C in the rabbit
Report No	298
Document No	Not reported
Guidelines followed in study	None reported
GLP/Officially recognised testing facilities	No, not conducted under GLP (not compulsory at time of study conduct)
Previous evaluation	Yes, supplementary in RAR (2015)
Short description of study design and observations	Male New Zealand White rabbits were administered a single oral dose of [^{14}C]-glyphosate (assumed to be manufactured by [redacted], purity not given) by gavage at dose levels within a range of 5.7 - 8.8 mg/kg bw. Three treatment groups of two or three animals were used each receiving the test compound labelled at one of three different sites. Urine, faeces and expired CO_2 were sampled at 12, 24, 48, 72, 96 and 120 h after dosing and assayed for radioactivity. At sacrifice after 120 h, blood and tissue samples (liver, kidney, muscle, fat, gut, spleen, heart, testes) were radiochemically analysed.
Short description of results	After a single oral gavage dosing to male rabbits 79 - 97 % of the dose was excreted within 120 h. Total urinary clearance was only 7 - 11 % in this period. Less than 1 % was recovered in expired air. The carcass retention at sacrifice was 1.87 - 4.85 % of the dose, however, most (74 - 98 %) could be accounted for by the gut and its content. In the other tissues, highest residues were found in the liver and kidneys; however, radioactivity in bone was not determined. The results indicate similarities and differences between species such as a lower urinary clearance rate and higher tissue retention in rabbits as compared to the rat.
Reasons for why the study is not considered relevant/reliable or not considered as key study	**Conclusion GRG:** Supplementary due to reporting deficiencies, Category 2a. Dose levels appear too low for meaningful and reliable residue analysis; however, this is the only dietary study on toxicokinetics of glyphosate in rabbits. As the report was received at a late stage of the dossier preparation no full summary was prepared. **Conclusion AGG:** The study is considered to be unacceptable due to the dose level being too low. In the DAR the study was considered supplementary and in the RAR (2015) this study was suggested to be useful as additional information. As previously discussed the results indicate similarities and differences between species with lower urinary clearance rate and higher tissue retention in rabbits compared to rats.

B.6.1.1.17. Study 17

Data point	CA 5.1.1/018
Report author	[redacted]
Report year	1998
Report title	Report on metabolism (absorption, tissue distribution & excretion with radio-active compounds - rats) of glyphosate
Report No	1087
Document No	Not reported
Guidelines followed in study	None reported
GLP/Officially recognised testing facilities	No, not conducted under GLP
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations	Two male Wistar rats per dose were administered unknown doses of [^{14}C]-glyphosate (0.56 x 10^7 and 1.11 x 10^7 Bq radioactivity in low and high dose, respectively) by feed. Urine, faeces and blood were collected at 2, 4, 8, 24 and 48 h and 7 and 15 days after dosing and assayed for radioactivity. At sacrifice after 15 days, the following tissues were radiochemically analysed: Brain, liver, spleen, heart, kidney, adrenals.
Short description of results	Glyphosate is poorly absorbed and levels of glyphosate in different tissues are negligible after 15 days of oral feeding.
Reasons for why the study is not considered relevant/reliable or not considered as key study	**Conclusion GRG:** Category 3b. The description of testing procedures and the reporting of results is very poor. Essential information like the dates of experimental work or the year when the study report was issued is lacking. Furthermore, there is no information on the amount of test substance administered (i.e. only the radioactivity applied is indicated). **Conclusion AGG:** The study is considered to be unacceptable due to a number of deficiencies including a low number of animals. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

B.6.1.2.1. Study 1

Data point	CA 5.1.2/001
Report author	[redacted]
Report year	2020
Report title	Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison
Report No	S19-04081
Document No	Not reported
Guidelines followed in study	Not available, but based on Commission Regulation (EU) No 283/2013, 5.1.1, in accordance with Regulation (EC) No 1107/2009 (ANNEX to SANCO/11802/2010 Rev. 7, as voted by the standing committee in July 2012)
Deviations from current test guideline	N/A

Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** No guideline is available for this study. However, the study was conducted in line with the recommendations made during the EFSA Workshop on *in vitro* comparative metabolism studies in regulatory pesticide risk assessment (EFSA Supporting publication 2019: EN-1618).

Executive summary

The objective of this study was to compare the metabolism of glyphosate after incubation with mixed gender cryo-preserved hepatocytes from human, rat, mouse and dog as well as with female rabbit cryo-preserved hepatocytes. 1 µM and 10 µM [^{14}C]-glyphosate were incubated separately with cryo-preserved hepatocytes and incubation buffer for 60 and 120 min and analysed by HPLC using radio detection.

The metabolic competence of the cryo-preserved hepatocytes was demonstrated using the positive controls testosterone and 7-hydroxycoumarin. 6β-hydroxytestosterone, 7-hydroxycoumarin sulphate and 7-hydroxycoumarin glucuronide were selected as a biological transformation marker compounds. All marker substances were detected after incubation in varying concentrations, proving the metabolic competence of the hepatocytes.

The dependence of the reaction on activated cryo-preserved hepatocytes was shown by the incubation of the test item (10 µM) for 120 min with inactivated cryo-preserved hepatocytes (negative control).

The determined metabolic transformation rates after incubation for 120 min with human, rat, dog and rabbit hepatocytes for [^{14}C]-glyphosate amounted up to 0.0 % for incubations with 1 µM and ≤1.0 % for incubations with 10 µM [^{14}C]-glyphosate, respectively. The rates after incubation for 120 min with mouse mixed cryo-preserved hepatocytes amounted up to 0.0 % for incubations with 1 µM and up to 1.56 % for incubations with 10 µM [^{14}C]-glyphosate, respectively.

Incubations of 1 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit hepatocytes revealed the parent substance and additional three potential metabolites during the incubation period. One unidentified substance (Unknown 1) detected in dog hepatocytes after an incubation time of 0 min showed the highest abundance (2.0 %). No major metabolites (>5 % of applied radioactivity; AR) were detected after an incubation time of 120 min in any species.

Incubations of 10 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit hepatocytes revealed the parent substance and additional seven potential metabolites during the incubation period. “Unknown 1” detected in mouse hepatocytes after an incubation time of 120 min showed the highest abundance (2.8 %). No major metabolites (>5 % AR) were detected after an incubation time of 120 min in any species.

Incubations of 1 and 10 µM [^{14}C]-glyphosate were being investigated for the metabolite AMPA, but no AMPA was detected in any species after an incubation time of 120 min.

No human unique metabolites were found. Therefore, all detected metabolites were only characterised based on their chromatographic behaviour and a metabolic pathway was not derived.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	[^{14}C]-Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]methyl)glycine
Lot/Batch #:	6848SXD008-2
Radiochemical purity:	98.3 % (HPLC)
Specific activity:	2060 MBq/mmol, 55.7 mCi/mmol
Stability of test compound:	Expiry date 2020-11-27 at ≤-18°C in a dark and dry place

2. Positive controls:

Phase 1:

Identification:	[^{14}C]-Testosterone
Position of radiolabel:	[4-^{14}C]-Testosterone
Lot/Batch #:	2379862
Radiochemical purity:	97 % (HPLC and TLC)
Specific activity:	1880 MBq/mmol, 50.8 mCi/mmol
Stability of test compound:	Expiry date 2020-01-16 at ≤-18°C in a dark and dry place

Phase 2:

Identification:	7-Hydroxycoumarin
Description:	Beige solid
Lot/Batch #:	BCBZ4431
Purity:	99.4 %
Stability of test compound:	Expiry date 2024-05-31 at ≤30°C in a dark and dry place

3. Reference substances:

Identification:	Glyphosate
Description:	White solid
Lot/Batch #:	BCHR 1037-02
Purity:	98.5 %
Stability of test compound:	Expiry date 2023-01-17 at ≤10°C in a dark and dry place
Identification:	Testosterone
Description:	White solid
Lot/Batch #:	SLBV0956
Purity:	100 %
Stability of test compound:	Expiry date 2020-06-30 at 5-30°C in a dark and dry place
Identification:	Aminomethylphosphonic acid (AMPA)
Description:	White solid
Lot/Batch #:	1002969

Purity:	98.7 %
Stability of test compound:	Expiry date 2024-06-05 at 1-10°C in a dark and dry place under inert gas
Identification:	6ß-Hydroxytestosterone
Description:	White solid
Lot/Batch #:	MKCD3716
Purity:	99 %
Stability of test compound:	Expiry date 2024-03-07 at ≤30°C in a dark and dry place
Identification:	7-Hydroxycoumarin sulphate (potassium salt)
Description:	White solid
Lot/Batch #:	0538267-5
Purity:	98.6 %
Stability of test compound:	Expiry date 2021-01-21 at ≤-18°C in a dark and dry place
Identification:	7-Hydroxycoumarin glucuronide (sodium salt)
Description:	White solid
Lot/Batch #:	023M3900V
Purity:	97 %
Stability of test compound:	Expiry date 2021-06-30 at ≤10°C in a dark and dry place

4. Negative control: Inactivated hepatocytes

5. Hepatocytes:

Species / Strain:	Human / - rat / Sprague Dawley mouse / CD-1 dog / Beagle rabbit / New Zealand White
Source:	XenoTech, USA and Prymacyt Cell Culture Technology GmbH, Germany
Sex:	All species male & female, except rabbit (female only)

B. STUDY DESIGN

Preparation of cryo-preserved hepatocytes

Number of donors accounted for 50 human female and 50 human male (age 8-74 for female and male), 12 rat female (age approx. 8-12 weeks) and 8 rat male (age approx. 8-12 weeks), 15 mouse female (age approx. 8-12 weeks) and 8 mouse male (age approx. 8-12 weeks), 1 dog female (age 5 years and 5 months) and 1 dog male (age 1 year and 3 months), and 3 rabbit female (age sexually mature). Mixed gender cryo-preserved hepatocytes were pooled in equal parts except for rat (12 female & 8 male). Cryo-preserved cell suspensions of human, rat, dog, mouse or rabbit hepatocytes were thawed and washed with the respective buffer media. After the last cleaning step, hepatocytes were re-suspended with Hepatocytes Plating Medium-Cryo (HPM-Cryo) to achieve 0.5 x 10^6 viable cells/mL.

Incubation of human, rat, mouse, dog and rabbit hepatocytes with the test substance [^{14}C]-glyphosate

The hepatocytes of humans, rats, dogs, mice and rabbits were incubated separately with [^{14}C]-glyphosate at concentration of 1 and 10 µM for 0, 60 and 120 min at 37 ± 2°C, in a final volume of 1000 µL, using a water bath with gentle shaking. [^{14}C]-Glyphosate in HPM-Cryo was incubated under the same conditions for 120 min as control. For each condition, duplicate samples were prepared and analysed at the beginning and after termination of the incubation.

The radioactivity in the supernatants was determined by LSC and investigated qualitatively and quantitatively by HPLC.

Incubation of human, rat, mouse, dog and rabbit hepatocytes with [^{14}C]-testosterone as positive control for Phase 1

The first positive control assay consisted of the evaluation of the ability of the cryo-preserved hepatocytes to metabolise testosterone. For this purpose, 10 µM [^{14}C]-testosterone was incubated with a volume of 500 µL cryo-preserved hepatocytes in HPM-Cryo. Incubations were run for 120 min.

The radioactivity in the supernatants was determined by LSC and investigated qualitatively and quantitatively by HPLC.

Incubation of human, rat, mouse, dog and rabbit hepatocytes with 7-hydroxycoumarin as positive control for Phase 2

The second positive control assay consisted of the evaluation of the ability of the cryo-preserved hepatocytes to metabolise 7-hydroxycoumarin. For this purpose, 3.244 mg/L solution of 7-hydroxycoumarin was incubated with a volume of 500 µL cryo-preserved hepatocytes in HPM-Cryo. Incubations were run for 120 min.

C. ANALYTICAL PROCEDURES

Radioactivity Measurement

Radioassaying of liquid samples was carried out by liquid scintillation counting (LSC) with automatic quench correction.

High Performance Liquid Chromatography (HPLC)

The test item [^{14}C]-glyphosate, the reference items, testosterone/7-hydroxycoumarin and their metabolites were qualitatively and quantitatively determined using HPLC. For all samples HPLC analysis was performed on an Agilent 1260 system with radiometric- and UV-detection.

To ensure the quantitative elution of the injected radioactivity from the HPLC-column, the radioactivity of aliquots was measured before and after injection.

The chromatographic recovery for [^{14}C]-glyphosate accounted for 96.7 - 100.1 % of the injected radioactivity indicating almost quantitative elution of the injected radioactivity.

Evaluation and presentation of the results

The cell vitality is calculated based on the ratio between counted living and total cells multiplied with the trypan blue dilution factor, the volume of the cell suspension and the factor of the Neubauer counting chamber according to following equation:

$$cell\ vitality = \emptyset\ Number\ of\ cells\ (living/total\ cells) \times trypan\ blue\ dilution\ factor \times volume\ cell\ suspension \times factor\ of\ Neubauer\ counting\ chamber$$

The relative percentages were calculated based on the area values of the HPLC profiles at the different incubation times according to following equation:

$$\%\ Relative\ P_i = \frac{(Area\ P_i)}{\sum Area\ P} \times 100$$

Where Area P_i is the area of a compound peak in the HPLC profile of a test sample, and ∑ Area P is the sum of the total radioactive peak areas in the chromatogram.

The metabolic transformation rate of the test item [^{14}C]-glyphosate over time was calculated according to the following equation:

$$\%\ Metabolic\ transformation\ rate$$

$$= 100 - \frac{\%\ Area\ test\ item\ incubation\ 0,\ 60\ or\ 120\ min}{\%\ Area\ reference\ item\ incubation\ 0\ min} \times 100\%$$

Where the relative percentages of the peak area of the test item after 0, 60 or 120 min incubation time were compared to the relative percentage measured at 0 min incubation time of the control incubation without hepatocytes.

The metabolic transformation rate of the positive control compounds [^{14}C]-testosterone and 7-hydroxycoumarin over time was calculated according to following equation:

$$\%\ Metabolic\ transformation\ rate$$

$$= 100 - \frac{\%\ Area\ positive\ control\ incubation\ 120\ min}{\%\ Area\ positive\ control\ incubation\ 0\ min} \times 100\%$$

Where the relative percentages of the peak area of the positive control after 0, 60 or 120 min incubation time were compared to the relative percentage measured at 0 min incubation time.

High Performance Mass Spectrometry (LC-MS)

HPLC combined with high resolution mass spectrometry was used to confirm the presence of the test item and available references. Samples were analysed by LC-MS using a Thermo Orbitrap QExactive Plus operating at a mass resolution of 70000 in positive and in negative ion mode. MS/MS product ion spectra were obtained using collision induced dissociation (CE 30) at a resolution of 17500.

As the resolution of the reference item AMPA in the analytical standard with UV detection has been insufficient, the presence of AMPA in the analytical standard was confirmed by HPLC combined with MS (full scan and negative ion mode) too. Therefore, the analytical standard of AMPA was measured using HPLC and the fraction where AMPA was assumed to retarded was isolated and then measured.

For the HPLC analysis a quantifiable radioactive peak (LOQ) was regarded as relevant having a signal at least higher than the possible statistical deviation of the background, which was set to 3 Sigma expected for testosterone incubates (6 Sigma). Sigma is the square root of the background part within the integration region. 3 Sigma therefore means 3 x the square root of the background part within the integration region. The limit of detection (LOD) was half this amount. Based on the estimated LOQ of the HPLC-analysis of incubates (0.10 - 1.97 % of the applied radioactivity), the profiling HPLC-methods ensured enough sensitivity for the detection of the test item, the reference item and their metabolites.

For Liquid Scintillation counting the detection limit was predefined on the base of the background radioactivity-counting rate, which was about 24.3 cpm. For the calculations, a reference point of 20 dpm was set as average background radioactivity. The limit of detection (LOD) was established as twice the average background radioactivity (40 dpm). The limit of quantification (LOQ) was established as three times the average background radioactivity (60 dpm). Single background subtraction and a quench and counting efficiency correction for transformation of gross counts (cpm) into net counts (dpm) were automatically performed by the instruments. Samples with individually measured values below 40 dpm (after correction for the background radioactivity) were not quantified and labelled as “n.q.” in the respective tables.

II. RESULTS AND DISCUSSION

A. CELL VITALITY

After reprocessing of the cryo-preserved hepatocytes, the cell vitality as number of living cells in ratio to total counted cells was determined for human, rat, mouse, dog and rabbit hepatocytes. The cell vitality amounted to 87, 83, 90, 92 and 90 % at the test item concentration of 1 µM and 84, 83, 86, 86 and 90 % at the test item concentration of 10 µM, respectively.

B. RECOVERY OF RADIOACTIVITY

The recovery of radioactivity at 0 min of the supernatants of treated hepatocytes ranged between 93.5 - 109.4 % of the applied radioactivity for the test item at 1 and 10 µM. After 60 min incubation the recoveries were between 93.1 - 103.4 % for the test item at 1 and 10 µM. After 120 min incubation the mean recoveries were between 90.7 - 106.4 % for the test item at 1 and 10 µM. The recovery for negative controls (incubation of 10 µM test item and inactivated hepatocyte) was between 90.4 – 106.4 % after 0 min and 90.2 - 101.2 % after 120 min.
The incubation of inactivated cryo-preserved hepatocytes with the test item (10 µM) proved that the biotransformation of the test item is dependent on the presence of activated cryo-preserved hepatocytes.

Table 6.1.2.1-37: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison [redacted] 2020): Recovery of radioactivity in supernatants after incubation of 1 and 10 µM [^{14}C]-glyphosate with cryo-preserved hepatocytes from human, rat, mouse, dog and rabbit and inactivated hepatocytes

Sample description	Incubation time [min]	Recovery of applied radioactivity [%]		
		Activated hepatocytes (1 µM)	Activated hepatocytes (10 µM)	Inactivated hepatocytes (10 µM)
Human	0	97.7	97.1	105.1
		100.5	99.0	98.3
Rat		102.9	101.6	100.6
		109.4	100.1	90.4
Mouse		93.5	100.7	106.4
		94.6	99.3	90.6
Dog		102.3	98.2	104.0
		101.2	100.2	104.0
Rabbit		103.3	96.5	98.8
		104.0	96.8	-
Human	60	98.5	96.4	-
		99.5	99.7	-
Rat		102.4	102.2	-
		99.7	101.5	-
Mouse		93.1	98.1	-
		93.7	99.0	-
Dog		102.5	97.7	-
		102.1	96.8	-
Rabbit		98.6	95.2	-
		103.4	93.3	-
Human	120	99.2	96.0	98.3
		101.4	98.0	101.2
Rat		99.1	103.5	90.2
		99.7	101.6	94.7
Mouse		90.7	98.3	99.3
		92.9	96.0	98.3
Dog		99.4	95.6	100.9
		101.9	97.5	100.1
Rabbit		100.8	94.8	94.4

Table 6.1.2.1-37: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Recovery of radioactivity in supernatants after incubation of 1 and 10 µM [^{14}C]-glyphosate with cryo-preserved hepatocytes from human, rat, mouse, dog and rabbit and inactivated hepatocytes

Sample description	Incubation time [min]	Recovery of applied radioactivity [%]		
		Activated hepatocytes (1 µM)	Activated hepatocytes (10 µM)	Inactivated hepatocytes (10 µM)
		106.4	96.2	94.3

Table 6.1.2.1-38: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison [redacted] 2020): Recovery of radioactivity in supernatants and [^{14}C]-testosterone and 6β-hydroxytestosterone concentrations in percent of total radioactive peak area after incubation of 10 µM [^{14}C]-testosterone with cryo-preserved hepatocytes from human, rat, mouse, dog and rabbit

Sample description	Incubation time [min]	Recovery of applied RA [%]	Area of [^{14}C]-testosterone [%*]	Area of 6β-hydroxy-testosterone [%]
Human	120	104.0	14.80	1.67
		101.9		
Rat		109.6	3.37	10.17
		110.5		
Mouse		106.8	23.80	10.89
		107.7		
Dog		102.4	37.95	6.40
		103.5		
Rabbit		103.6	2.91	ND
		108.9		

RA radioactivity; samples were taken after 120 min of incubation and analysed by LSC
ND not detected
* % peak area

C. BIOTRANSFORMATION OF [^{14}C]-GLYPHOSATE

The highest transformation rate (1.71 %) was observed after 0 minutes of incubation at 10 µM [^{14}C]-glyphosate with mouse cryo-preserved hepatocytes. The results are compiled in the table below.

Table 6.1.2.1-39: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Concentration of [^{14}C]-glyphosate in percent of total radioactive peak area and metabolic transformation [^{14}C]-glyphosate after incubation with rats, mouse, dog, rabbit and human hepatocytes at 1 and 10 µM for 60 and 120 min

Species	Conc. [µM]	Area of [^{14}C]-glyphosate [%]			Metabolic transformation rate [%]*,**		
		Incubation time [min]			Incubation time [min]		
		0	60	120	0	60	120
Rat	**1**	99.20	100.00	100.00	0.00	0.00	0.00
	10	98.76	98.46	98.65	0.00	≤1.00	≤1.00
Mouse	**1**	100.0	100.00	99.10	0.00	0.00	0.00
	10	97.03	97.71	97.18	1.71	1.02	1.56
Dog	**1**	98.03	100.00	100.00	≤1.00	0.00	0.00
	10	99.09	99.84	99.20	0.00	0.00	0.00

Rabbit	**1**	99.38	100.00	99.76	0.00	0.00	0.00
	10	98.21	98.72	98.56	≤1.00	≤1.00	≤1.00
Human	**1**	99.77	100.00	100.00	0.00	0.00	0.00
	10	98.87	97.81	97.74	0.00	≤1.00	≤1.00

* The metabolic transformation rate was calculated on the basis of the % area of [^{14}C]-glyphosate of the control incubation without cryo-preserved hepatocytes.
** Metabolic transformation rates between 0 and 1.00 % are reported as ≤1.00 %

D. METABOLITES AFTER BIOTRANSFORMATION OF [^{14}C]-GLYPHOSATE

Incubations of 1 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit hepatocytes revealed the parent substance and three further unknown substances/peaks during the incubation period. One peak "Unknown 1" showed the highest abundance (2.0 %) in dog hepatocytes after an incubation time of 0 min. The highest concentration of another not further identified peak "Unknown 2" was detected in rat hepatocytes after an incubation time of 0 min. No major metabolites (>5 % AR) were detected after incubation of hepatocytes of any species with 1 µM [^{14}C]-glyphosate at any time point.

Incubations of 10 µM [^{14}C]-glyphosate with human, rat, dog, mouse and rabbit cryo-preserved hepatocytes revealed the parent substance and additional six unknown substances during the incubation period. "Unknown 1" detected in mouse hepatocytes after an incubation time of 120 min showed the highest abundance (2.8 %) but was already detected at 2.6 % after 0 min of incubation. Further unknown substances/peaks were detected in low concentrations. Most of those were however already present before incubation, decreased by time or were just observed at one time point. No major metabolites (>5 % AR) were detected after incubation of hepatocytes of any species with 10 µM [^{14}C]-glyphosate at any time point.

All detected unknown substances in human hepatocytes were also detected in rat, mouse, dog or rabbit hepatocytes. Therefore, no human specific metabolite was observed.

All detected unknown substances were only characterised based on their chromatographic behaviour and a metabolic pathway was not derived. The metabolite AMPA which was characterised based on LC-MS/MS was not detected in any sample.

Table 6.1.2.1-40: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Distribution of the applied radioactivity [%] after incubation of rats, mouse, dog, rabbit and human hepatocytes with 1 and 10 µM [^{14}C]-glyphosate after 0, 60 and 120 min

Species	**Metabolite**	**1 µM**			**10 µM**		
		Incubation time [min]			**Incubation time [min]**		
		0	**60**	**120**	**0**	**60**	**120**
Human	Unknown 1	-	-	-	0.8	2.2	2.0
	Unknown 2	0.2	-	-	0.3	-	-
	Unknown 4	-	-	-	-	-	0.3
	Glyphosate	99.8	100.0	100.0	98.9	97.8	97.7
Rat	Unknown 1	-	-	-	0.9	0.9	1.2
	Unknown 2	0.8	-	-	-	-	-
	Unknown 4	-	-	-	0.3	0.3	0.2
	Unknown 5	-	-	-	-	0.4	-
	Glyphosate	**99.2**	**100.0**	**100.0**	**98.8**	**98.5**	**98.7**
Mouse	Unknown 1	-	-	0.3	2.6	1.9	2.8
	Unknown 2	-	-	0.3	-	-	-
	Unknown 3	-	-	-	-	0.2	-
	Unknown 4	-	-	-	0.35	0.2	-
	Unknown 5	-	-	0.3	-	-	-

	Glyphosate	**100.0**	**100.0**	**99.1**	**97.0**	**97.7**	**97.2**
Dog	Unknown 1	2.0	-	-	0.3	-	0.3
	Unknown 2	-	-	-	0.4	0.2	0.3
	Unknown 4	-	-	-	0.2	-	-
	Unknown 6	-	-	-	-	-	0.3
	Glyphosate	**98.0**	**100.0**	**100.0**	**99.1**	**99.8**	**99.2**
Rabbit	Unknown 1	0.6	-	-	1.8	1.3	1.4
	Unknown 2	-	-	0.2	-	-	-
	Glyphosate	**99.4**	**100.0**	**99.8**	**98.2**	**98.7**	**98.6**

E. BIOTRANSFORMATION OF POSITIVE CONTROLS

[^{14}C]-Testosterone

6β-Hydroxytestosterone was selected as a biological transformation marker compound and detected after incubation with [^{14}C]-testosterone for 120 min with human, rat, dog and mouse cryo-preserved hepatocytes. After incubation with rabbit cryo-preserved hepatocytes 6β-hydroxytestosterone was not detected. The relative peak area in percent of [^{14}C]-testosterone was low (2.91 %) while the recovery of applied radioactivity of testosterone for rabbits was high. This suggests that [^{14}C]-testosterone was further transformed. The highest metabolic activity was thus detected for rabbit female cryo-preserved hepatocytes. The concentration of [^{14}C]-testosterone was 2.91 % after 120 min. The concentration of [^{14}C]-testosterone in human hepatocytes was 14.80 % after 120 min. The highest [^{14}C]-testosterone concentration after 120 min was 37.95 % in dog hepatocytes.

7-Hydroxycoumarin

7-Hydroxycoumarin sulphate was found after incubation with 7-hydroxycoumarin for 120 min with rat and dog cryo-preserved hepatocytes. 7-Hydroxycoumarin glucuronide was found after incubation with 7-hydroxycoumarin for 120 min with human, rat, mouse, dog and rabbit cryo-preserved hepatocytes.
The lowest concentration of 7-hydroxycoumarin, considered as marker for the highest metabolic activity, was measured for rat cryo-preserved hepatocytes and amounted to 10.01 % of 7-hydroxycoumarin after 120 min. 25.43 % of the total applied radioactivity was identified as 7-hydroxycoumarin after 120 min incubation with human cryo-preserved hepatocytes while 54.44 % of the totally applied radioactivity was identified as the respective glucuronide conjugate.

Table 6.1.2.1-41: Metabolic stability and profiling of [^{14}C]-Glyphosate in hepatocytes from human, rat, mouse, dog and rabbit for inter-species comparison ([redacted] 2020): Recovery of radioactivity after incubation of rat, mouse, dog, rabbit and human hepatocytes with 10 µM [^{14}C]-testosterone and hydroxycoumarin, respectively after 120 min

Species	**6β-hydroxytestosterone**	**[^{14}C]-testosterone**	**7-hydroxy coumarin glucuronide**	**7-hydroxy coumarin**	**7-hydroxy coumarin sulphate**
	% Relative peak area				
Human	1.67	14.80	54.44	25.43	-
Rat	10.17	3.37	23.11	10.01	6.97
Mouse	10.89	23.80	33.87	12.94	-
Dog	6.40	37.95	49.50	11.33	10.94
Rabbit	-	2.91	47.31	27.37	-

III. CONCLUSIONS

Incubations with cryo-preserved hepatocytes of all tested species exhibited a very similar biotransformation pattern of the applied [^{14}C]-glyphosate. The highest biotransformation rate of [^{14}C]-glyphosate was detected after incubation of 10 µM with cryo-preserved hepatocytes from mouse (1.71 %). The biotransformation rate after incubations with human, rat, dog and rabbit hepatocytes was ≤1.00 %.
All detected unknown substances were characterised based on their chromatographic behaviour. No AMPA was detected in hepatocytes of any species after an incubation time of 120 min with 1 and 10 µM [^{14}C]-glyphosate, respectively. No human unique metabolites were detected.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The *in-vitro* metabolite profile of 1 µM and 10 µM [^{14}C]-glyphosate was determined after incubation with cryo-preserved hepatocytes from human, rat, dog, mouse and rabbit for 0, 60 and 120 min at 37°C and analysis by HPLC and liquid scintillation counting. Based on the estimated limit of quantitation (LOQ) of the HPLC-analysis of incubates, the profiling HPLC methods ensured enough sensitivity for the detection of the test item, the reference item and their metabolites. The metabolic capability of the used cryo-preserved hepatocytes batches was proven by control incubations with 7-hydroxycoumarine and [^{14}C]-testosterone. Negative controls with inactivated hepatocytes confirmed the stability of the test item in the test medium.

After incubation of cryo-preserved hepatocytes of all tested species with 1 and 10 µM [^{14}C]-glyphosate no relevant metabolism was observed in any species. At least 97 % of the applied radioactivity was identified as glyphosate. The remaining proportion of the applied radioactivity was detected as several smaller peaks. The structure of the respective substances was not elucidated. The highest radioactivity of such a unknown substance reached up to 2.8 % of applied radioactivity for "Unknown 1" in mouse hepatocytes after 120 minute incubation. However, this substance was already detected at 2.6 % in mouse hepatocytes without incubation time, indicating that this substance is not a metabolite of mouse hepatocytes. Further unknown substances/peaks were detected in lower concentrations for all species. Most of those were however already present before incubation, decreased by time without appearance of another unknow substance or were just observed at one time point. Thus, most of the identified unknow substances are considered to be not metabolites of glyphosate. No AMPA was detected in hepatocytes of any species after an incubation time of 120 min with 1 and 10 µM [^{14}C]-glyphosate, respectively.
Taken together, no major metabolites (>5 % AR) were detected after incubation of hepatocytes of any species with 10 µM [^{14}C]-glyphosate at any time point and no human unique metabolites were detected.

Assessment and conclusion by RMS:
Agreed with the conclusions by the applicant. There are no human specific metabolites for glyphosate detected.

B.6.2. ACUTE TOXICITY

B.6.2.1. Oral

B.6.2.1.1. *Study 1*

Data point:	CA 5.2.1/001
Report author	[redacted]
Report year	2014
Report title	Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method
Report No	41401853
Document No	Not reported
Guidelines followed in study	OECD 420 (2001) Method B1 bis (EC) No. 440/2008

Deviations from current test guideline (OECD 420, 2001)	None
Previous evaluation	Accepted in RAR (2015)
GLP/Officially recognised testing facilities	Neither the GLP compliance statement, nor the quality assurance statement was signed.
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: The study is not considered to be acceptable due to the missing signatures on the GLP compliance and quality assurance.

Executive Summary

The acute oral toxicity of glyphosate technical was investigated in female rats (5 animals) of the Wistar strain. Following a sighting test at a dose level of 2000 mg/kg bw, an additional four fasted female animals were given a single dose of test item, as a dispersion/suspension in dimethyl sulphoxide by oral gavage to each animal at a dose level of 2000 mg/kg bw and constant dose volume of 10 mL/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study.

No clinical signs of systemic toxicity were noted in the first treated animal during the observation period, whereas hunched posture was noted during the day of dosing in four additional treated animals. All animals showed expected body weight gains over the observation period. No mortality occurred. No abnormal necropsy findings were noted. The acute oral LD_{50} was: LD_{50}, oral, female rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	04062014
Purity:	85.79%
Stability of test compound:	4 June 2016 (expiry date)

2. Vehicle and/or positive control: Dimethyl sulphoxide

3. Test animals:

Species:	Rat
Strain:	RccHanTM:Wistar
Source:	[redacted]
Age:	8 – 12 weeks
Sex:	Female
Weight at dosing:	141 – 171 g
Acclimation period:	At least 5 days
Diet/Food:	2014C Teklad Global Rodent diet supplied by Harlan Laboratories UK Ltd., Oxon, UK, *ad libitum* (except overnight fast prior to dosing and 3-4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	Maximum of 4 animals / cage in suspended solid-floor polypropylene cages furnished with woodflakes

Environmental conditions:	Temperature:	19 – 25 °C
	Humidity:	30 – 70%
	Air changes:	at least 15 per hour
		12-hour light / dark cycle (light during 06:00 – 18:00)

B. STUDY DESIGN AND METHODS

In life dates: 2014-07-10 to 2014-07-30

Animal assignment and treatment:
Female Wistar (RccHanTM:Wist) strain rats were supplied by [redacted] On receipt the animals were randomly allocated to cages. The females were nulliparous and non-pregnant. The body weight variation did not exceed ±20 % of the body weight of the initially dosed animal. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food was allowed throughout the study.
For the purpose of the study the test item was freshly prepared, as required, as a dispersion/suspension in dimethyl sulphoxide. Dimethyl sulphoxide was used because the test item did not dissolve/suspend in distilled water or arachis oil BP. The test item was formulated within two hours of being applied to the test system. It is assumed that the formulation was stable for this duration.
No analysis was conducted to determine the homogeneity, concentration or stability of the test item formulation. This is an exception with regard to GLP and has been reflected in the GLP compliance statement.
Using available information on the toxicity of the test item, 2000 mg/kg bw was chosen as the starting dose (one animal) and administered at a dosing volume of 10 mL/kg. In the absence of toxicity at a dose level of 2000 mg/kg bw, an additional group of four animals was treated. All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. Clinical observation was made 0.5, 1, 2, and 4 hours after dosing and then daily for fourteen days. Morbidity and mortality checks were made twice daily. Individual body weights were recorded on day 0 (the day of dosing) and on days 7 and 14.
At the end of the observation period the animals were killed by cervical dislocation. All animals were subjected to gross necropsy. This consisted of an external examination and opening of the abdominal and thoracic cavities. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the 14-day observation period after administration.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were noted in the initial treated animal during the observation period. Hunched posture was noted during the day of dosing in four additional treated animals (see table below).

Table B.6.2.1.1-1: Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method [redacted] 2014): Summary of clinical observations

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Time after treatment		0.5 h	1 h	2 h	4 h	Day 1 - 14
Total animals examined	**n**	5	5	5	5	5
Clinical sign	**n**	4	2	1	1	0
Hunched posture	n	4	2	1	1	0
Single animals observations						
Animal No.						
1-0		*	*	*	*	*
2-0		A	*	*	*	*
2-1		A	A	A	A	*
2-2		A	A	*	*	*
2-2		A	*	*	*	*

* = No abnormalities detected, A = Hunched posture

C. BODY WEIGHT

All animals showed expected gains in body weight over the observation period. Individual body weights are provided in the table below.

Table B.6.2.1.1-1: Glyphosate: Acute Oral Toxicity in the Rat – Fixed Dose Method [redacted] 2014): Body weight and body weight gain

Dose level	**2000 mg/kg bw**				
Sex	**Females**				
Day	**0**	**7**	**14**	**0-7**	**7-14**
Animal No.§	**Body weight [g]**			**Body weight gain [g]**	
1-0	171	194	203	23	9
2-0	167	192	211	25	19
2-1	160	180	191	20	11
2-2	141	155	165	14	10
2-3	142	160	178	18	18
Mean ± SD	**156.2 ± 14.0**	**176.2 ± 18.0**	**189.6 ± 18.6**	**20 ± 4.3**	**13.4 ± 4.7**

§ = animal No. of females

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No macroscopic findings were recorded at the scheduled necropsy. No tissues were retained.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw in females.

Assessment and conclusion by applicant:

The study is in concordance with the current OECD 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

It is agreed with the applicant that the study was conducted according to OECD 420 (2001). Nevertheless, the study is not considered acceptable for evaluation since signatures are missing in the study report.

This conclusion is not in line with the previous evaluation where the study was accepted (RAR, 2015).

B.6.2.1.2. *Study 2*

Data point:	CA 5.2.1/002
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure)
Report No	10/218-001P
Document No	Not reported
Guidelines followed in study	OECD 425 (2008) OPPTS 870.1100 (2002)
Deviations from current test guideline (OECD 425, 2008)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

Executive Summary
Two limit tests with different dose levels were performed. As there were no clinical signs or macroscopic findings observed at the dose level of 2000 mg/kg bw a limit test at a higher dose level (5000 mg/kg bw) was requested by the Sponsor, data from the animals treated at 2000 mg/kg bw were archived in the raw data without any further reporting.
In an acute oral toxicity study (limit test), a group of three, fasted, 10-11 week old, RjHan:WI female rats was given a single oral dose of Glyphosate Technical (96.3 % w/w Glyphosate technical) in 0.5 % carboxymethylcellulose (CMC) at a concentration of 5000 mg/kg bw and administered at a dosing volume of 10 mL/kg bw. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter. Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and on days 7 and 14. All animals were necropsied and examined macroscopically.
Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs.
No deaths occurred during the study. No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw. There was no treatment related changes in the body weights. The body weights of the animals were within the range commonly recorded for this strain and age. No test item-related macroscopic findings were observed. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 5000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	Technical, dry white powder
Lot/Batch #:	569753(BX20070911)
Purity:	96.3%
Stability of test compound:	Stable under storage conditions (room temperature range < 30 °C), recertification date end August 2011

2. Vehicle and/or positive control: 0.5% Carboxymethylcellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	RjHan:WI
Source:	[redacted]
Age:	10 – 11 weeks
Sex:	Female
Weight at dosing:	228 – 231 g
Acclimation period:	At least 21 days
Diet/Food:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany, *ad libitum* (except for pre-dose fast and 3 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	Individually in Type II. polypropylene / polycarbonate cages with Lignocel Bedding for Laboratory Animals

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 – 70%
Air changes: 15 – 20 / hour
12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2011-01-20 to 2011-02-10

Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 10 – 11 week old, RjHan:WI female rats was given a single oral dose of glyphosate technical (96.3 % w/w glyphosate technical) at a concentration of 5000 mg/kg bw by gavage. The test substance was diluted in vehicle (0.5 % carboxymethylcellulose) and administered at a dosing volume of 10 mL/kg bw. Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. Treatment of an animal at the next dose was only performed when no significant clinical signs were noted in the previous animal. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3, 4 and 6 hours after treatment on day 1 and once daily for 14 days thereafter. Body weights were recorded on day -1 (prior to removal of food), day 0 (prior to administration) and on days 7 and 14. All animals were exsanguinated under pentobarbital anaesthesia at the end of the observation period, necropsied and examined macroscopically.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.

C. BODY WEIGHT
The body weight of the animals was within the range commonly recorded for this strain and age. Individual body weights are listed below in the table below.

Table B.6.2.1.2-1: Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) [redacted] 2011): Body weight development and gain of rats administered glyphosate

Dose group	**5000 mg/kg bw**							
Sex	**Females**							
Day	**-1**	**0**	**7**	**15**	**-1 to 0**	**0 to 7**	**7 to 14**	**-1 to 14**
Animal No.	**Body weight (g)**				**Body weight gain (g)**			
3512	242	229	252	266	-13	23	14	24
3213	247	228	253	263	-19	25	10	16
3511	241	231	245	257	-10	14	12	16
Mean (± SD)	**243.3 ± 3.2**	**229.3 ± 1.5**	**250.0 ± 4.4**	**262.0 ± 4.6**	**-14.0 ± 4.6**	**20.7 ± 5.9**	**12.0 ± 2.0**	**18.7 ± 4.6**

D. NECROPSY
No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in accordance with the current OECD guideline 425 (2008). The outcome can be therefore reported

as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate (technical) in female rats is above 5000 mg/kg bw.

B.6.2.1.3. *Study 3*

Data point:	CA 5.2.1/003
Report author	[redacted]
Report year	2010
Report title	Acute Oral Toxicity Study of Glyphosate TC in Rats
Report No	24874
Document No	Not reported
Guidelines followed in study	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method, 2001) and OPPTS 870.1100
Deviations from current test guideline (OECD 423, 2001)	The animals were slightly younger (7 weeks) than requested by the OECD GD. This is considered to have no impact on the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

Executive Summary
The test substance, glyphosate TC, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #: 2009051501

Purity: 96.4%

Stability of test compound: Expiry date: 2011-05-15

2. Vehicle and/ or positive control: 0.8 % aqueous hydroxypropylmethylcellulose

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 7 weeks
Sex:	Female
Weight at dosing:	171 – 192 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70 % 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2010-10-15 to 2010-11-10

Animal assignment and treatment:
Two groups of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study. On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance (see table below).

Table B.6.2.1.3-1: Acute Oral Toxicity Study of Glyphosate TC in Rats [redacted] 2010): Body weight development

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Step	**Animal No.**	**Body weight (g)**		**Body weight gain (%)**	**Body weight (g)**	**Body weight gain (%)**
		Day 1	**Day 8**		**Day 15**	
1	1	188	219	+16.5	230	+22.3
	2	180	217	+20.6	227	+26.1
	3	183	212	+15.8	226	+23.5
	Mean (± SD)	**183.7 ± 4.0**	**216.0 ± 3.6**	**+17.6**	**227.7 ± 2.1**	**+24**
2	4	171	198	+15.8	208	+21.6

Table B.6.2.1.3-1: Acute Oral Toxicity Study of Glyphosate TC in Rats [redacted] 2010): Body weight development

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Step	**Animal No.**	**Body weight (g)**		**Body weight gain (%)**	**Body weight (g)**	**Body weight gain (%)**
		Day 1	**Day 8**		**Day 15**	
	5	192	218	+13.5	226	+17.7
	6	187	214	+14.4	223	+19.3
	Mean (± SD)	**183.3 ± 11.0**	**210.0 ± 10.6**	**+14.6**	**219 ± 9.6**	**+19.5**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than2000 mg/kg bw.

Assessment and conclusion by applicant:

The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

It is agreed with the applicant's conclusion. The acute oral LD_{50} is above 2000 mg/kg bw.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.1.4. *Study 4*

Data point:	CA 5.2.1/004
Report author	[redacted]
Report year	2010
Report title	Acute Oral Toxicity Study of Glyphosate TC in Rats
Report No	24602
Document No	Not reported
Guidelines followed in study	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method, 2001) and OPPTS 870.1100 (1998)
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

Executive Summary
The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 2000 mg/kg bw

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate TC
Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3%
Stability of test compound:	Expiry date: May 2011
2. Vehicle and/ or positive control:	0.8% aqueous hydroxypropylmethylcellulose
3. Test animals:	
Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Approx. 7 – 8 weeks
Sex:	Female
Weight at dosing:	154 – 196 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70% 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-10-26 to 2009-11-24

Animal assignment and treatment:
Two groups of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6, and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study. On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table B.6.2.1.4-1: Acute Oral Toxicity Study of Glyphosate TC in Rats [redacted] 2010): Body weight development

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Step	**Animal No.**	**Body weight (g)**		**Body weight gain (%)**	**Body weight (g)**	**Body weight gain (%)**
		Day 1	**Day 8**		**Day 15**	
1	1	160	207	+29.4	228	+42.5
	2	154	189	+22.7	212	+37.7
	3	159	188	+18.2	205	+28.9
	Mean (± SD)	**157.7 ± 3.2**	**194.7 ± 10.7**	**+23.5**	**215.0 ± 11.8**	**+36.3**
2	4	171	202	+18.1	217	+26.9
	5	185	209	+13.0	226	+22.2
	6	196	252	+28.6	284	+44.9
	Mean (± SD)	**184.0 ± 12.5**	**221.0 ± 27.1**	**+20.1**	**242.3 ± 36.4**	**+31.7**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} is above 2000 mg/kg bw.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.1.5. *Study 5*

Data point:	CA 5.2.1/005
Report author	[redacted]
Report year	2009
Report title	Acute Oral Toxicity Study of Glyphosate TC in Rats
Report No	23910

Document No	Not reported
Guidelines followed in study	EC method B.1 tris (2004/73/EC), OECD 423 (ATC method, 2001) and OPPTS 870.1100 (1998)
Deviations from current test guideline (OECD 423, 2001)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

Executive Summary
The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White, solid
Lot/Batch #:	20080801
Purity:	98.8%
Stability of test compound:	Expiry date: 2010-08-01

2. Vehicle and/or positive control: 0.8 % aqueous hydroxypropylmethylcellulose gel

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	50 – 51 days
Sex:	Female
Weight at dosing:	167 – 186 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 3 animals were kept in MAKROLON cages (type III plus) with granulated textured wood as bedding material

Environmental conditions: Temperature: 22 ± 3 °C
Rel. humidity: 40 – 70%
12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-04 to 2009-03-04

Animal assignment and treatment:
Two groups of three fasted females received the test material at a single dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and thereafter in weekly intervals up to the end of the study. On Day 14 after dosing, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance (see table below).

Table B.6.1.2.5-1: Acute Oral Toxicity Study of Glyphosate TC in Rats [redacted] 2009): Body weight development

Dose group		**2000 mg/kg bw**				
Sex		**Females**				
Step	**Animal No.**	**Body weight (g)**		**Body weight gain (%)**	**Body weight (g)**	**Body weight gain (%)**
		Day 1	**Day 8**		**Day 15**	
1	1	167	205	+22.8	213	+27.5
	2	182	213	+17.0	229	+25.8
	3	171	200	+17.0	216	+26.3
	Mean (± SD)	**173.3 ± 7.8**	**206.0 ± 6.6**	**+18.9**	**219.3 ± 8.5**	**+26.5**
2	4	172	209	+21.5	221	+28.5
	5	186	221	+18.8	224	+20.4
	6	183	214	+16.9	225	+23.0
	Mean (± SD)	**180.3 ± 7.4**	**214.7 ± 6.0**	**+19.1**	**223.3 ± 2.1**	**+23.8**

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:

The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} is above 2000 mg/kg bw.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.1.6. *Study 6*

Data point:	CA 5.2.1/006
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Acute Oral Toxicity Study in Rats
Report No	C22864
Document No	Not reported
Guidelines followed in study	OECD 423 (2001) Commission Regulation (EC) No 440/2008 (2008), method B.1 tris
Deviations from current test guideline (OECD 423, 2001)	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 to be acceptable.

Executive Summary
The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female HanRcc: WIST (SPF) rats when administered as a gavage dose at a level of 2000 mg/kg bw. No mortality occurred during the study. No clinical signs were observed during the course of the study. There was no effect on body weight gain. No macroscopic findings were recorded at necropsy. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66%
Stability of test compound:	(Stable under storage conditions) Expiry date: July 2010

2. **Vehicle and/ or positive control:** Purified water
3. **Test animals:**

Species:	Rat
Strain:	HanRcc: WIST (SPF)

Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	181.0 – 198.7 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseaugst / Switzerland) *ad libitum* (except for the overnight fasting period prior to intubation and approximately 3-4 hours post dose)
Water:	Tap water, *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Shill AG, 4132 Muttenz / Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70% Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-13 to 2009-02-03 and 2009-02-05

Animal assignment and treatment:
Two groups of three fasted females each received the test material at a dose level of 2000 mg/kg bw by oral gavage. The dosing volume was 10 mL/kg bw. Observations for mortality and clinical signs of toxicity were made at least five times on the day of dosing (Day 1) and at twice daily thereafter during days 2 – 15. Individual body weights were recorded just prior to dosing and on Days 8 and 15. On Day 15 after dosing, each animal was euthanised by CO_2 asphyxiation. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed during the course of the study.

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for this strain and age.

Table B.6.2.1.6-1: Glyphosate Technical: Acute Oral Toxicity Study in Rats [redacted] 2009): Body weight development

Dose group	**2000 mg/kg bw**		
Sex	**Females**		
Animal No.	**Body weight (g)**		
	Day 1	**Day 8**	**Day 15**
1	182.3	194.4	197.6
2	181.0	190.8	202.4
3	198.7	209.8	220.8
Mean (± SD)	**187.3 ± 9.9**	**198.3 ± 10.1**	**206.9 ± 12.2**
4	198.6	221.0	227.1
5	183.8	204.8	216.5
6	192.2	206.8	222.0

Table B.6.2.1.6-1: Glyphosate Technical: Acute Oral Toxicity Study in Rats ([redacted] 2009): Body weight development

Dose group	**2000 mg/kg bw**		
Sex	**Females**		
Animal No.	**Body weight (g)**		
	Day 1	**Day 8**	**Day 15**
Mean (± SD)	**191.5 ± 7.4**	**210.8 ± 8.9**	**221.9 ± 5.3**

D. NECROPSY

No macroscopic findings were recorded at necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate (technical) in female rats is above 2000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.7. *Study 7*

Data point:	CA 5.2.1/007
Report author	[redacted]
Report year	2009
Report title	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats
Report No	12170-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1100 Equivalent to OECD 425 (2008)
Deviations from current test guideline (OECD 425, 2008)	Humidity was in the range of 33 – 89 % instead of 30 – 70 %. It is not expected that this deviation has an impact on the study outcome.
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 to be acceptable.

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident by Day 8. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.40%
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat albino
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	8 weeks
Sex:	Female
Weight at dosing:	160 – 187 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 89% Air changes: 10 – 12 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2008-11-11 to 2008-11-27

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The dosing volume was 12.5 mL/kg bw. Observations for mortality and clinical / behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, animals were sacrificed, subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs in one animal included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident at Day 8 (see table below).

Table B.6.2.1.7-1: Glyphosate: Acute Oral Toxicity Study (UDP) In Rats [redacted] 2009): Clinical observations

Dose level	**5000 mg/kg bw (12.5 mL/kg bw)**
Sex	**Female**

Time after dosing		Day 0*			Days							
Animal No.	**Parameter**	**1st**	**2nd**	**3rd**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8 - 14**
291	Salivation	-	s	s	s	s	-	-	-	-	-	-
	Piloerection	-	-	-	m	m	m	s	s	s	s	-
	Diarrhea	-	-	-	s	m	m	m	m	-	-	-
	Polyuria	-	-	-	-	m	m	m	m	-	-	-
	Activity decrease	-	-	-	-	-	-	-	s	-	-	-
292	Appeared normal at each observation											
293	Appeared normal at each observation											

* = Observations were performed at three time points on the day of dosing (Day 0)
s = slight, m = moderate

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance. Individual and mean body weights see table below.

Table B.6.2.1.7-2: Glyphosate: Acute Oral Toxicity Study (UDP) In Rats [redacted] 2009): Body weights

Animal No.	Body Weights (g)		
	Day 0	**Day 7**	**Day 14**
291	187	203	231
292	168	199	204
293	160	183	194
Mean ± SD	**171.7 ± 13.9**	**195.0± 10.6**	**209.7 ± 19.1**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance to the current OECD guideline 425 (2008). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate (technical) in female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.8. *Study 8*

Data point:	CA 5.2.1/008
Report author	[redacted]
Report year	2008
Report title	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical
Report No	[redacted]-3996.305.475.07 (including amendment 1)
Document No	Not reported
Guidelines followed in study	OECD guideline 423 (2001)
Deviations from current test guideline (OECD 423 (2001)	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 to be acceptable.

Executive Summary
The test substance, Glyphosate technical, was tested for acute oral toxicity in female albino rats using a stepwise procedure. The test item was administered orally at single dose levels of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50} cut-off value, oral, female rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate Technical

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat albino
Strain:	Wistar Hannover
Source:	[redacted]
Age:	8 - 9 weeks
Sex:	Female
Weight at dosing:	172 - 205 g
Acclimation period:	6 days
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum* except for fasting overnight before dosing
Water:	Filtered drinking water, *ad libitum*
Housing:	Groups of three rats per cage were held in polypropylene rodents cages with wire mesh tops and bedding material
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: min. 10/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-09-12 to 2008-06-11

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 2000 mg/kg bw by oral gavage in a stepwise manner. As no mortality was observed, a second group of three fasted female rats was dosed in the same manner. Observations for mortality and clinical/behavioural signs of toxicity were made once within the

first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were recorded just prior to dosing (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was euthanised by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed in females treated with 2000 mg/kg bw.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table B.6.2.1.8-1: Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical [redacted] 2008): Body weight and body weight gain.

Dose group		**2000 mg/kg bw**					
Sex		**Females**					
Time after treatment		**Day 0**	**Day 7**	**Day 14**	**Day 0 to 7**	**Day 7 to 14**	**Day 0 to 14**
Step	**Animal No.**	**Body weight (g)**			**Body weight gain g)**		
1	25	180	203	213	23	10	33
	26	205	229	239	24	10	34
	27	203	230	243	27	13	40
	Mean (± SD)	**196.0 ± 13.9**	**220.7 ± 15.3**	**231.7 ± 16.3**	**24. 7 ± 2.1**	**11.0 ± 1.7**	**35.7 ± 3.8**
2	28	183	206	208	23	2	25
	29	186	210	217	24	7	31
	30	172	198	206	26	8	34
	Mean (± SD)	**180.3 ± 7.4**	**204. 7 ± 6.1**	**210.3 ± 5.9**	**24.3 ± 1.5**	**5.7 ± 3.2**	**30.0 ± 4.6**

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate (technical) in female rats is above 2000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.9. *Study 9*

Data point:	CA 5.2.1/009
Report author	[redacted]
Report year	2007

Report title	Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure)
Report No	B02755
Document No	Not reported
Guidelines followed in study	OECD 425 (2001) US EPA OPPTS 870.1100 (2002) Japanese MAFF 12 NohSan No. 8147 (2001)
Deviations from current test guideline (OECD 425, 2008)	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 to be acceptable.

Executive Summary
In an acute oral toxicity study (limit test), a group of three, fasted, 11 week old, HanRcc:WIST (SPF), female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) in purified water at a concentration of 5000 mg/kg bw and administered at a dosing volume of 10 mL/kg bw.
The animals were examined daily during the acclimatisation period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2 – 15. Mortality/viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15. Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.
Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs.
All animals survived until the end of the study period. Slightly ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading. The body weight of the animals was within the range commonly recorded for this strain and age. No macroscopic findings were recorded at the scheduled necropsy. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical material
Description:	Technical, white powder
Lot/Batch #:	0507
Purity:	96.1%
Stability of test compound:	Re-certification date August 2008. Stable under storage conditions (room temperature range 20 ± 5 °C, protected from light and humidity)

2. Vehicle and/or positive control: Purified water (deionised water processed and treated by the PURELAB Option-R unit which links four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation)

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]

Age:	11 weeks
Sex:	Female
Weight at dosing:	183.0 – 188.9 g
Acclimation period:	5 – 7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum* (except for pre-dose fast and 3 hours after dosing)
Water:	Community tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70% Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2006-12-06 to 2006-12-26

Animal assignment and treatment:
In an acute oral toxicity study, a group of three, fasted, 11 week old, HanRcc:WIST (SPF), female rats was given a single oral dose of glyphosate technical material (96.1 % w/w glyphosate acid) at a concentration of 5000 mg/kg bw by gavage. The test substance was diluted in vehicle (purified water) and dosed at a volume dosage of 10 mL/kg bw. Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg bw. As no mortality or significant clinical signs were observed, two additional animals were sequentially dosed at 5000 mg/kg bw such that a total of 3 animals were tested. No mortalities were observed; therefore, the study was terminated.
The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2 – 15. Mortality / viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2 – 15. Body weights were recorded on day -1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15. All animals were killed at the end of the observation period by carbon dioxide asphyxiation, necropsied and examined macroscopically.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
Slight ruffled fur was noted in all animals from the 30-minute reading to the 5-hour reading and persisted in one animal until test day 3. Hunched posture was also noted in the animals from the 1- or 2-hour reading to the 5-hour reading (see table below).

Table B.6.2.1.9-1: Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure) ([redacted] 2007): Summary of observations during the study.

Dose group		**5000 mg/kg bw**							
Sex		**Females**							
Time after treatment		0.5 h	1 h	2 h	3 h	5 h	2 days	3 days	4-15 days
Total animals examined	**n**	3	3	3	3	3	3	3	3
Clinical sign	**n**	3	3	3	3	3	1	1	0
Ruffled fur	n	3	3	3	3	3	1	1	0

Hunched posture n	0	1	3	3	3	0	0	0
Single animals observations								
Animal No.								
1	s	sh	sh	sh	sh	s	s	*
2	s	s	sh	sh	sh	*	*	*
3	s	s	sh	sh	sh	*	*	*

* = No abnormalities detected, s = slight ruffled fur, h = hunched posture

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for this strain and age. The individual body weights are listed below in the table below.

Table B.6.2.1.9-2: Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure) [redacted] 2007): Individual body weights recorded during the study.

Dose group	**5000 mg/kg bw**			
Sex	**Females**			
	Day -1	**Day 1**	**Day 8**	**Day 15**
Animal No.	**Body weight (g)**			
1	185.4	183.0	190.9	195.9
2	198.8	184.1	205.9	215.8
3	193.7	188.9	204.8	211.6
Mean (± SD)	**192.6 ± 6.8**	**185.3 ± 3.1**	**200.5 ± 8.4**	**207.8 ± 10.5**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY

No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:

The study is in accordance with the current OECD guideline 425 (2008). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate (technical) in female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.10. *Study 10*

Data point:	CA 5.2.1/010
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP05068): Acute Oral Toxicity Study in Rats
Report No	B02272
Document No	Not reported
Guidelines followed in study	Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute oral toxicity studies. Guideline 2"1-1 Notification 12 NohSan No. 8147, as partly revised

	in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005. Directive 2004173/EC, 8.1 tris "Acute Oral Toxicity-Acute Toxic Class Method", April 29,2004. OECD Guidelines for the Testing of Chemicals, Number 423 "Acute Oral Toxicity – Acute Toxic Class Method", adopted 17 December 2001
Deviations from current test guideline (OECD 423, 2001)	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 to be acceptable.

Executive Summary
The test substance, glyphosate, was evaluated for its acute oral toxicity potential in female rats when administered as a 2000 mg/kg bw gavage dose. No mortality occurred during the study. The only clinical sign observed was slightly ruffled fur. There was no effect on body weight gain and no macroscopic findings were recorded at necropsy.
The median lethal dose of glyphosate technical (NUP 05068) after single oral administration to female rats, observed over a period of 14 days is: LD_{50} (female rat) > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1%
Stability of test compound:	Stable under storage conditions. Expiry date: 14 September 2008

2. Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	160 – 187 g
Acclimation period:	5 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland), *ad libitum* except for 18 – 19 hours before dosing and 3 hours after dosing.
Water:	Tap water, *ad libitum*
Housing:	In groups of three in Makrolon type-4 cages with wire mesh tops and standard softwood bedding ('Lignocel' Schill AG, CH-4132 Muttenz/Switzerland)

Environmental conditions: Temperature: 22 ± 3 °C
Humidity: 30 – 70%
Air changes: 10 – 15 / hour
12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2006-12-12 to 2007-01-04

Animal assignment and treatment:
The animals received a single dose of the test item by oral gavage administration at 2000 mg/kg bw after being fasted for approximately 18 – 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing. The dosing volume was 10 mL/kg bw. Observations for mortality and viability: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2 – 15.
Body weights: On test days 1 (prior to administration), 8 and 15.
Clinical signs: Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2 – 15. All abnormalities were recorded.
Necropsy: All animals were sacrificed at the end of the observation period and subjected to macroscopic examinations.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
A slightly ruffled fur was noted in all six animals treated at 2000 mg/kg bw after dosing. Otherwise no clinical signs were observed in any animal at any observation time (see table below).

Table B.6.2.1.10-1: GLYPHOSATE TECHNICAL (NUP05068): Acute oral toxicity study [redacted] 2007): Summary of clinical observations

Dose group		**2000 mg/kg bw**					
Sex		**Females**					
Time after treatment		0.5 h	1 h	2 h	3 h	5 h	Day 2 - 15
Total animals examined	**n**	6	6	6	6	6	6
Clinical sign	**n**	0	3	6	6	0	0
Slightly ruffled fur	n	0	3	6	6	0	0
Single animals observations							
Animal No.							
1		*	*	A	A	*	*
2		*	*	A	A	*	*
3		*	*	A	A	*	*
4		*	A	A	A	*	*
5		*	A	A	A	*	*
6		*	A	A	A	*	*

* = No abnormalities detected, A = slightly ruffled fur

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance (table below).

Table B.6.2.1.10-2: GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study [redacted] 2007): Body weight development

Dose group	**2000 mg/kg bw**
Sex	**Females**

Table B.6.2.1.10-2: GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study [redacted] 2007): Body weight development

Time after treatment		Day 1	Day 8	Day 15
Step	**Animal No.**	**Body weight (g)**		
1	1	189.6	206.3	220.7
	2	189.4	209.8	213.6
	3	184.4	203.6	207.6
2	4	175.0	197.9	198.9
	5	186.2	208.8	219.7
	6	187.6	211.6	222.8
	Mean ± SD	**185.4 ± 5.4**	**206.3 ± 5.0**	**213.9 ± 9.2**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical in female rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant: The study is in concordance with the current OECD guideline 423 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate (technical) in female rats is above 2000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.11. *Study 11*

Data point:	CA 5.2.1/011
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats
Report No	15274
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1100 (2002) OECD 425 (2001)
Deviations from current test guideline (OECD 425, 2008)	Humidity and air changes were not reported. It is not expected that this has a significant impact on the study outcome.
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 to be acceptable.

Executive Summary

The test substance, glyphosate acid technical, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals

recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, female rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate Acid Technical

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat albino
Strain:	Sprague-Dawley derived
Source:	[redacted]
Age:	11 weeks
Sex:	Female
Weight at dosing:	222 – 235 g
Acclimation period:	21 or 23 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum* except for overnight fasting before dosing and 3 – 4 hours after dosing
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week
Environmental conditions:	Temperature: 19 – 23 °C Humidity: not reported Air changes: not reported 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2004-05-04 to 2004-05-20

Animal assignment and treatment:
A group of three fasted females received the test material at a dose level of 5000 mg/kg bw by oral gavage in a sequential manner according to the up-and-down procedure (limit test). The test substance was administered as a 50 % w/w suspension in distilled water. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanised by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs noted for all animals included diarrhea, ano-genital and facial staining, and/or reduced fecal volume. All animals recovered by Day 4 and appeared active and healthy for the remainder of the 14-day observation period (see table below).

11-1: Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats [redacted] 2005): Clinical observations after treatment

Dose group	**5000 mg/kg bw**							
Sex	**Females**							
Time after treatment	**1 h**	**2 h**	**3 h**	**5 h**	**Day 1**	**Day 2**	**Day 3**	**Day 4-14**
Total animals n examined	3	3	3	3	3	3	3	3
Clinical sign n	0	0	1	2	3	2	1	0
Diarrhea n	0	0	0	1	0	0	0	0
Anogenital staining n	0	0	0	2	2	2	1	0
Facial staining n	0	0	1	0	0	0	0	0
Reduced fecal volume n	0	0	0	0	1	0	0	0
Single animals observations								
Animal No.								
4765	*	*	*	AD	A	*	*	*
4856	*	*	*	A	A	A	*	*
4857	*	*	C	*	B	A	A	*

* = No abnormalities detected, A = anogenital staining, B = reduced fecal volume, C = facial staining, D = diarrhea

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see table below).

Table B.6.2.1.11-2: Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats [redacted] 2005): Body weight development during the study

Dose group	**5000 mg/kg bw**		
Sex	**Females**		
Time after treatment	**Day 0**	**Day 7**	**Day 14**
Animal No.	**Body weight (g)**		
4765	234	247	269
4856	235	247	271
4857	222	239	263
Mean ± SD	**230 ± 7.2**	**244.3 ± 4.6**	**167.7 ±4.2**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate acid technical in female rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance with the current OECD guideline 425 (2008). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate acid (technical) in female rats is

above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.12. *Study 12*

Data point:	CA 5.2.1/012
Report author	[redacted]
Report year	1999
Report title	NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test
Report No	7907
Document No	Not reported
Guidelines followed in study	US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)
Deviations from current test guideline (OECD 420, 2001)	Animals of both sexes used, humidity and air changes not specified, purity of only 62%. It is not expected that these deviations have a significant impact on the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** In contrast to the applicant, the study is considered to be acceptable.

Executive Summary
NUP5a99 62 % glyphosate MUP was administered to ten healthy rats by oral gavage at the dose of 5000 mg/kg bw. All animals survived and gained weight during the study. Following administration, most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2 and appeared active and healthy for the remainder of the study. Gross necropsy findings at terminal sacrifice were unremarkable. Based on the results of this study, the single dose acute oral LD_{50} of the test substance is: LD_{50}, oral, male and female rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	NUP5a99 62 % glyphosate MUP
Description:	clear viscous amber liquid
Lot/Batch #:	Drum Sample E
Purity:	62% (isopropylamine glyphosate)
Stability of test compound:	No data available

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[redacted]
Age:	Not specified
Sex:	5 males and 5 females

Weight at dosing:	Young adult / males 227 – 254 grams and females 178 – 200 grams at experimental start
Acclimation period:	14 days
Diet/Food:	Purina Rodent Chow #5012 (Fasting approximately 17.25 h before and 3.5 h after dosing)
Water:	Tap water, *ad libitum*
Housing:	singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22 – 24 °C Humidity: not specified Air changes: not specified 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1999-08-03 – 1999-08-17

Animal assignment and treatment:
Prior to dosing, a group of animals was fasted for approximately 17.25 hours by removing feed from their cages. After weighing and clinical examination, ten (five male and five female) healthy rats were selected for test. Individual doses were calculated based on the initial body weights, taking into account the specific gravity (determined by PSL) of the test substance. Each animal received 5000 mg/kg bw of the test substance via gavage. Feed was replaced approximately 3.5 hours after dosing. The day of administration was considered Day zero of the study. Animals were weighed prior to test substance administration (initial) and again on Days 7 and 14 (termination). Clinical signs were recorded at 1, 3 and 22 hours post-dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and product safety labs central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea and coma. All rats were euthanised via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2. Details on clinical observations are provided in the table below.

B.6.2.1.12-1: NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test [redacted] 1999): Summary of clinical observations

Dose group		**5000 mg/kg bw**							
Sex		**Males**				**Females**			
Time after treatment		1 h	3 h	22 h	Day 1-14	1 h	3 h	22 h	Day 1-14
Total animals examined	**n**	5	5	5	5	5	5	5	5
Clinical sign	**n**	0	0	0	0	0	1	4	0
Anogenital staining	n	0	0	0	0	0	1	4	0
Soft feces	n	0	0	0	0	0	0	1	0
Diarrhea	n	0	0	0	0	0	0	1	0
Single animals observations									
Animal No.§									
7733 (7738)		*	*	*	*	*	*	A	*
7734 (7739)		*	*	*	*	*	A	A	*
7735 (7740)		*	*	*	*	*	*	*	*
7736 (7741)		*	*	*	*	*	*	AB	*
7737 (7742)		*	*	*	*	*	*	AC	*

§ = animal No. of males and (females), * = No abnormalities detected, A = anogenital staining, B = soft feces, C

Table B.6.2.1.12-1: NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test [redacted] 1999): Summary of clinical observations

Dose group	**5000 mg/kg bw**							
Sex	**Males**				**Females**			
Time after treatment	1 h	3 h	22 h	Day 1-14	1 h	3 h	22 h	Day 1-14

= diarrhea

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance. Individual and group mean body weights are listed below in the table below.

Table B.6.2.1.12-2: NUP5a99 62 % glyphosate MUP: Acute oral toxicity study in rats – Limit test [redacted] 1999): Body weight development after treatment with glyphosate

Dose level	**5000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**14**	**0**	**7**	**14**
Animal No.§	**Body weight (g)**					
7733 (7738)	240	313	364	178	203	244
7734 (7739)	254	324	355	184	203	251
7735 (7740)	242	321	371	200	224	247
7736 (7741)	244	310	329	180	206	239
7737 (7742)	227	300	347	183	220	250
Mean (± SD)	**241.4 ± 9.7**	**313.6 ± 9.5**	**353.2 ± 16.3**	**185.0 ± 8.7**	**211.2 ± 10.0**	**246.2 ± 4.9**

§ = animal No. of males and (females), note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The single dose acute oral LD_{50} of NUP5a99 62 % glyphosate MUP is greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study was performed according to a national guideline similar to the current OECD guideline 420 (2001). The study outcome was therefore considered valid. Nevertheless, the study is considered as supportive due to the low purity of the test substance. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
In contrast to what the applicant is stating, merely the fact that the purity of the test item is low does not classify the study as supportive only. Furthermore, the study itself is considered valid although it was not conducted according to an OECD guideline.
The acute oral LD_{50} of NUP5a99 62 % glyphosate MUP in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.13. *Study 13*

Data point:	CA 5.2.1/013
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Acute Oral Toxicity Study In Rats
Report No	[redacted] P/4660

Document No	Not reported
Guidelines followed in study	OECD 401 (1987) US EPA OPPTS 870.1100 (2002)
Deviations from current test guideline (OECD 420, 2001).	Animals of both sexes used. Clinical observation was performed twice after treatment, once within 2 hours instead of the first 30 minutes. The deviations are not expected to have a significant impact on the study outcome.
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 to be acceptable.

Executive Summary
In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid in deionised water and observed for 15 days. None of the animals died. There were no signs of systemic toxicity and no treatment-related findings at examination *post mortem*. All animals gained weight during the study. Based on the study, the following is concluded: LD_{50}, oral, male and female rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Acid
Description:	Technical, white solid
Lot/Batch #:	P24
Purity:	95.6%
Stability of test compound:	The test substance was used within the expiry date

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Age:	Young adult
Sex:	Male and female
Weight at dosing:	233 – 260 g (males), 197 – 225 g (females)
Acclimation period:	At least 6 days
Diet/Food:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK, *ad libitum* (except overnight immediately prior to dosing).
Water:	Mains water, *ad libitum*
Housing:	5/cage, sexes separately in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 – 70% Air changes: Approximately 25 – 30 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-03-16 to 1995-03-30

Animal assignment and treatment:
In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid by gavage. The test substance was diluted in deionised water. The volume of the dose was calculated for each animal according to its weight at the time of dosing and a standard volume of 10 mL/kg bw of the dosing preparation was administered. Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made daily, up to day 15. The animals were weighed on the day before dosing (day -1), immediately before dosing (day 1) and on days 3, 5, 8 and 15. All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
There were no signs of systemic toxicity.

C. BODY WEIGHT
All animals lost weight initially due to the pre-dose fast, but all had exceeded their initial weight by day 3, and apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study (see table below).

Table B.6.2.1.13-1: Glyphosate Acid: Acute Oral Toxicity Study In Rats [redacted] 1996): Individual body weights

Dose	**5000 mg/kg bw**											
Sex	**Males**						**Females**					
Animal No.§	**Day -1**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**	**Day -1**	**Day 1**	**Day 3**	**Day 5**	**Day 8**	**Day 15**
	Body weight (g)											
11 (67)	255	237	265	283	304	359	197	177	208	209	214	232
12 (68)	260	232	261	279	300	339	225	203	232	230	227	243
13 (69)	233	210	243	263	285	330	222	201	233	247	262	272
14 (70)	254	228	265	278	303	345	216	192	224	230	239	244
15 (71)	260	233	270	280	304	333	218	197	223	227	244	255
Mean (± SD)	**252.4 ± 11.2**	**228.0 ± 10.6**	**260.8 ± 10.4**	**276.6 ± 7.8**	**299.2 ± 8.1**	**341.2 ± 11.5**	**215.6 ± 11.0**	**194.0 ± 10.4**	**224.0 ± 10.0**	**228.6 ± 13.5**	**237.2 ± 18.1**	**249.2 ± 15.1**

§ = animal No. of males and (females)

D. NECROPSY
Red or mottled areas in the lung or red areas in the thymus were seen in three males and two females. These are common spontaneous findings in rats of this age and strain and are considered not to be treatment-related. Individual findings at necropsy are listed in the table below.

Table B.6.2.1.13-2: Glyphosate Acid: Acute Oral Toxicity Study In Rats [redacted] 1996): Necropsy findings

Animal No. and sex	**Organ**	**Observation**
11 M	Lung	Red area/s: (moderate) on all lobes and surfaces
12 M	Thymus	Red area/s: covering most of right lobe
13 M	-	-
14 M	Lung	Mottled: (moderate) red

Table B.6.2.1.13-2: Glyphosate Acid: Acute Oral Toxicity Study In Rats [redacted] 1996): Necropsy findings

15 M	-	-
67 F	Lung	Mottled: (slight) red
68 F	-	-
69 F	-	-
70 F	Lung	Red area/s: (slight) on all lobes and surfaces
71 F	-	-

- = No findings

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate acid in male and female rats was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in accordance with the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate acid in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.14. *Study 14*

Data point:	CA 5.2.1/014
Report author	[redacted]
Report year	1995
Report title	An Acute Toxicity Study of MON 0139 by Oral Administration in Mice
Report No	B-3101 [redacted] study no. XX-95-205
Document No	Not reported
Guidelines followed in study	JMAFF 59 NohSan No. 4200 (January 28, 1985)
Deviations from current test guideline (OECD 420, 2001)	Animals of both sexes used, animals 7 weeks old instead of 8 and 12 weeks, 9 instead of 5 animals per sex were used in the sighting study, no fasting after dosing. These deviations are not expected to significantly impact the study outcome.
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 to be acceptable.

Executive Summary

The test substance, MON 0139, was evaluated for its acute oral toxicity potential in male and female Crj:CD-1(ICR) strain SPF mice when administered as a gavage dose at 5000 mg/kg bw. No mortality occurred during the study. No clinical signs of toxicity were observed. There was a slight tendency toward retardation of body weight gain as compared with the control group for males from 7 days after administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be: LD_{50}, oral, mouse > 5000 mg/kg body weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	MON 0139
Description:	Light yellow viscous solution
Lot/Batch #:	LBRV-11092
Purity:	62.34% (isopropyl amine salt of glyphosate)
Stability of test compound:	Stable under room temperature, expiry date July, 1996

2. Vehicle and/or positive control: Water for injection

3. Test animals:

Species:	Mouse
Strain:	Crj:CD-1(ICR)
Source:	[redacted]
Age:	6 weeks; 7 weeks at administration
Sex:	Male and female
Weight at dosing:	♂ 31.1 – 34.5 g; ♀ 22.2 – 26.2 g
Acclimation period:	Approximately 1 week
Diet/Food:	CRF-1 pelleted diet, sterilised by radiation (Oriental Yeast Co., Ltd.), *ad libitum* except during fasting prior to dosing
Water:	Tap water; *ad libitum*
Housing:	Plastic cages with wood chip bedding in groups of 5 (groups of 5 or 6 during quarantine/acclimation)
Environmental conditions:	Temperature: 23 ± 3 ºC Humidity: 50 ± 20% Air changes: 11 – 13 per hour Light cycle: 12 hour illumination per day

B. STUDY DESIGN AND METHODS

In life dates: 1995-08-16 – 1995-09-06

Animal assignment and treatment:

Preliminary study:
During the quarantine/acclimatization period, a preliminary study was conducted using 9 males and 9 females. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage, adjusting the dose volume according to each dose level. Three male and female animals were dosed with 1000, 2000, or 5000 mg/kg bw. The animals were fed again after administration, and had free access to water throughout the experimental period.

Main Study:
In the preliminary study, no deaths were observed in either sex in any of the dose groups. Based on these results, the dose level of 5000 mg/kg bw was selected for the main test. The animals were ranked by individual body weights and randomly assigned to groups so as to ensure the homogeneity of group means as far as possible. The animals were fasted for approximately 4 hours prior to administration and the test article was administered once orally, by gavage. The animals in the 5000 mg/kg bw group and control group were given 4.1 mL/kg body weight of test article and 'water for injection', respectively. Each group consisted of 5 animals per sex. Animals were fed again after administration, and had free access to water throughout the experimental period. The animals were observed frequently for the first 6 hours after administration, and then once daily for 14 days for mortality, signs of toxicity and abnormalities. Body weights were recorded prior to fasting, immediately before dosing, and on days 1, 2, 3, 7, 10, and 14 after dosing. A gross necropsy was performed on all animals at the

time of terminal sacrifice at the end of the 14-day observation period.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed.

C. BODY WEIGHT

In males, a slight tendency toward retardation of body weight gain as compared with the control group was observed in the 5000 mg/kg bw group from 7 days after administration (see table below). In females, no compound-related changes were observed in the 5000 mg/kg bw group.

Table B.6.2.1.14-1: Acute Toxicity Study of MON 0139 by Oral Administration in Mice [redacted] 1995): Body weight development in mice after treatment with glyphosate

Day		**0[a]**	**0**	**1**	**2**	**3**	**7**	**10**	**14**	**0-14**
Sex		**Males**								
Dose level (mg/kg bw)	**Animal No.[§]**	**Body weight [g]**								**Bw gain [g]**
0	1001	32.6	31.3	33.7	33.8	34.1	37.0	35.2	37.3	6.0
	1002	33.3	31.1	33.8	34.4	34.6	35.9	36.5	37.6	6.5
	1003	33.3	32.2	33.5	34.2	34.3	36.4	40.1	37.4	5.2
	1004	36.0	34.5	35.1	36.1	36.3	38.2	39.2	41.1	6.6
	1005	36.6	34.1	36.5	37.3	37.7	39.2	39.7	40.4	6.3
Mean		**34.4**	**32.6**	**34.5**	**35.2**	**35.4**	**37.3**	**38.1**	**38.8**	**6.1**
± SD		**± 1.8**	**± 1.6**	**± 1.3**	**± 1.5**	**± 1.6**	**± 1.3**	**± 2.2**	**± 1.8**	**± 0.6**
5000	2001	34.4	32.0	31.2	30.8	31.2	33.5	33.5	35.3	3.3
	2002	34.6	31.7	34.1	34.1	34.5	34.7	35.2	35.8	4.1
	2003	34.9	33.3	35.3	36.2	36.8	37.3	38.1	39.4	6.1
	2004	35.0	33.4	34.9	33.8	34.0	34.2	35.4	37.7	4.3
	2005	35.3	33.7	35.3	35.7	35.7	36.0	37.1	38.2	4.5
Mean		**34.8**	**32.8**	**34.2**	**34.1**	**34.4**	**35.1**	**35.9**	**37.3**	**4.5**
± SD		**0.4**	**± 0.9**	**± 1.7**	**± 2.1**	**± 2.1**	**± 1.5**	**± 1.8**	**± 1.7**	**± 1.0**
			Females							
0	**1101**	25.2	24.3	25.3	24.9	24.4	25.2	25.5	26.4	2.1
	1102	25.3	23.9	25.5	26.0	24.5	26.3	26.9	26.6	2.7.
	1103	26.7	25.2	27.3	27.2	25.2	25.8	26.4	26.9	1.7
	1104	27.2	26.2	26.7	26.8	26.7	28.2	29.2	29.2	3.0
	1105	25.9	24.4	26.8	27.1	25.4	27.5	27.4	29.8	5.4
Mean		**26.1**	**24.8**	**26.3**	**26.4**	**25.2**	**26.6**	**27.1**	**27.8**	**3.0**
± SD		**± 0.9**	**± 0.9**	**± 0.9**	**± 1.0**	**± 0.9**	**± 1.2**	**± 1.4**	**± 1.6**	**± 1.4**
5000	**2101**	22.9	22.2	23.3	24.0	23.7	24.7	24.5	25.8	3.6
	2102	24.6	23.7	23.5	23.8	23.1	24.8	24.2	23.4	-0.3
	2103	25.6	25.1	26.7	26.7	26.2	28.1	29.3	31.4	6.3
	2104	26.0	24.9	26.3	26.5	25.9	25.6	27.1	28.7	3.8
	2105	27.0	25.1	28.1	28.2	26.0	27.5	27.4	27.5	2.4
Mean		**25.2**	**24.2**	**25.6**	**25.8**	**25.0**	**26.1**	**26.5**	**27.4**	**3.2**
± SD		**± 1.6**	**± 1.3**	**± 2.1**	**± 1.9**	**± 1.5**	**± 1.6**	**± 2.1**	**± 3.0**	**± 2.4**

§ = animal No. of males and (females), a = prior to fasting

D. NECROPSY
No abnormalities were observed.

III. CONCLUSIONS

The acute oral LD_{50} of the test material (MON 0139; 62.34% (isopropyl amine salt of glyphosate)) in male and female mice was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed according to a national guideline similar to the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of MON 0139 (62.34 % isopropyl amine salt of glyphosate) in male and female mice is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.15. *Study 15*

Data point:	CA 5.2.1/015
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute Oral Toxicity Study In Rats
Report No	[redacted]94-0134
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations from current test guideline (OECD 420, 2001):	At dosing, animals were 6 weeks old instead of 8 to 12 weeks. In addition, both sexes were used. After administration the first observation was after 1 h instead of during the first 30 minutes. Temperature 23 ºC (± 3 ºC) instead of 22 ºC (± 3 ºC). The deviations are not expected to significantly impact the study outcome.
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 to be acceptable.

Executive Summary
The test substance, glyphosate technical (HR-001), was evaluated for its acute oral toxicity potential in Sprague Dawley rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included decreased spontaneous motor activity and salivation 1 and 3 hours after administration. No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, rats > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	940908-1
Purity:	95.68%
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: 0.5 % carboxymethyl-cellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crj:CD), SPF
Source:	[redacted]
Age:	5 weeks; 6 weeks at administration
Sex:	Males and females
Weight at dosing:	♂ 168 – 179 g; ♀ 125 – 142 g
Acclimation period:	7 days
Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for an overnight fast before dosing and about 3 h after dosing
Water:	Tap water, *ad libitum*
Housing:	Wire-mesh stainless steel cages in groups of 5 animals/sex/cage
Environmental conditions:	Temperature: 23 ± 3 °C Humidity: 55 ± 15% Air changes: 12 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment
A group of five fasted rats per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made after 1, 3, and 6 hours on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was euthanised under ether anaesthesia and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Decreased spontaneous motor activity was observed in five males and three females as well as salivation in one male. These signs were observed at 1 and 3 hours after the administration (see table below).

Table B.6.2.1.15-1: HR-001: Acute Oral Toxicity Study In Rats [redacted] 1995): Summary of clinical observations

Dose group		**5000 mg/kg bw**							
Sex		**Males**				**Females**			
Time after treatment		1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
Total animals examined	**n**	5	5	5	5	5	5	5	5
Clinical sign	**n**	4	3	0	0	2	3	0	0

Table B.6.2.1.15-1: HR-001: Acute Oral Toxicity Study In Rats [redacted] 1995): Summary of clinical observations

Dose group		**5000 mg/kg bw**							
Sex		**Males**				**Females**			
Time after treatment		1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
Decreased spontaneous motor activity	n	4	3	0	0	2	3	0	0
Salivation	n	1	0	0	0	0	0	0	0
Single animals observations									
Animal No.§									
1 (101)		A	A	*	*	A	A	*	*
2 (102)		A	*	*	*	A	A	*	*
3 (103)		A	*	*	*	*	*	*	*
4 (104)		AB	A	*	*	*	*	*	*
5 (105)		*	A	*	*	*	A	*	*

§ = animal No. of males and (females), * = No abnormalities detected, A = decreased spontaneous motor activity, B = salivation

C. BODY WEIGHT

No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration (Day 0). Individual and group mean body weights are provided in the table below.

Table B.6.2.1.15-2: HR-001: Acute Oral Toxicity Study In Rats [redacted] 1995): Body weight development

Dose group	**5000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**14**	**0**	**7**	**14**
Animal No.§	**Body weight [g]**					
1 (101)	178	266	320	136	187	216
2 (102)	172	251	310	129	169	191
3 (103)	171	246	297	125	165	181
4 (104)	168	239	296	129	180	214
5 (105)	179	265	322	142	187	211
Mean (± SD)	**173.6 ± 4.7**	**253.4 ± 11.8**	**309 ± 12.3**	**132.2 ± 6.8**	**177.6 ± 10.2**	**202.6 ± 15.7**

§ = animal No. of males and (females)
Note: Standard deviation was not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical (HR-001) in male and female rats was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. The study is in concordance with the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate technical (HR-001) in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.16. *Study 16*

Data point:	CA 5.2.1/016
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute Oral Toxicity Study In Mice
Report No	[redacted]94-0133
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), JMAFF 59 NohSan 4200 (1985), US EPA (1984)
Deviations from current test guideline (OECD 420, 2001)	2 hours fasting instead of 3-4 hours, animals were 6 weeks old at start of administration instead of 8 to 12 weeks. After administration the first observation was after 1 h instead of during the first 30 minutes. It is not expected that these deviations have a significant impact on the study outcome.
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 to be acceptable.

Executive Summary
The test substance, glyphosate technical (HR-001), was evaluated for its acute oral toxicity potential in ICR mice when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included decreased spontaneous motor activity, sedation and crouching position 1 and 3 hours after administration. There were no body weight losses at 14 days after administration when compared with the body weights on the day of administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, mice > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	940908-1
Purity:	95.68%
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control: 0.5 % carboxymethyl-cellulose (CMC)

3. Test animals:

Species:	Mice
Strain:	ICR (Crj:CD-1)
Source:	[redacted]
Age:	6 weeks
Sex:	Males and females
Weight at dosing:	♂ 29.4 – 32.7 g; ♀ 22.8 – 25.8 g
Acclimation period:	7 days

Diet/Food:	Pellet Diet MF (Oriental Yeast Co., Japan), *ad libitum* except for approx. 2 h before dosing, and 3 h after dosing
Water:	Tap water, *ad libitum*
Housing:	Aluminium cages with wire-mesh floors in groups of 5 animals/sex/cage
Environmental conditions:	Temperature: 23 ± 3 °C Humidity: 55 ± 15% Air changes: 12 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-01-24 to 1995-02-07

Animal assignment and treatment:
A group of five fasted mice per sex received the test material at a dose level of 5000 mg/kg bw by oral gavage (limit test). The dosing volume was 20 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made after 1, 3, and 6 hours on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, animals were sacrificed and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
Decreased spontaneous motor activity was observed in one male and one female as well as sedation and crouching position in another male. These signs were observed at 1 and 3 hours after the administration. A summary of all recorded clinical signs is provided in the table below.

Table B.6.2.1-16-1: HR-001: Acute Oral Toxicity Study In Mice [redacted] 1995): Clininal signs after dosing

Dose group		**5000 mg/kg bw**							
Sex		**Males**				**Females**			
Time after treatment		1 h	3 h	6 h	Day 1-14	1 h	3 h	6 h	Day 1-14
Total animals examined	**n**	5	5	5	5	5	5	5	5
Clinical sign	**n**	2	1	0	0	1	0	0	0
Decreased spontaneous motor activity	n	1	0	0	0	1	0	0	0
Sedation	n	1	1	0	0	0	0	0	0
Crouching	n	1	1	0	0	0	0	0	0
Single animals observations									
Animal No.§									
1 (101)		*	*	*	*	*	*	*	*
2 (102)		*	*	*	*	*	*	*	*
3 (103)		A	*	*	*	A	*	*	*
4 (104)		*	*	*	*	*	*	*	*
5 (105)		BC	BC	*	*	*	*	*	*

§ = animal No. of males and (females), * = No abnormalities detected, A = decreased spontaneous motor activity, B = sedation, C = crouching

C. BODY WEIGHT
7 days after administration, a slight body weight loss (0.5 g) was observed in one male when compared with the body weight on the day of administration. No body weight losses were recorded in any animal 14 days after the administration. A summary of the recorded bodyweights is provided in the table below.

Table B.6.2.1-16-2: HR-001: Acute Oral Toxicity Study In Mice [redacted] 1995): Body weight development

Dose group	**5000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**14**	**0**	**7**	**14**
Animal No.[§]	**Body weight [g]**					
1 (101)	29.4	30.7	32.3	22.8	24.7	26.8
2 (102)	30.3	32.0	32.5	24.1	26.1	27.9
3 (103)	30.1	31.5	33.4	23.8	25.5	25.9
4 (104)	31.8	33.1	36.3	23.2	24.8	25.9
5 (105)	32.7	32.2[$]	33.6	25.8	26.5	28.9
Mean (± SD)	**30.9 ± 1.4**	**31.9 ± 0.9**	**33.6 ± 1.6**	**23.9 ± 1.2**	**25.5 ± 0.8**	**27.1 ± 1.3**

§ = animal No. of males and (females)
$ = Body weight loss when compared with the pre-treatment value.
Note: Standard deviation was not given in the study report. Values were calculated retrospectively.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate technical (HR-001) in male and female mice was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. The study is in concordance with the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate technical (HR-001) in male and female mice is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.17. *Study 17*

Data point:	CA 5.2.1/017
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD_{50} tests with [redacted] Glyphosate acid technical in rats, limit test"
Report No	00917
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), EPA Pesticide Assessment Guidelines, Subdivison F, Series 81-1 (1984)
Deviations from current test guideline (OECD 420, 2001)	5 animals of both sexes used. Air changes not specified. Body weights only recorded once prior to start of study and not once a week during the study. Individual body weights were not reported. Individual clinical signs were not reported, however it was mentioned that no clinical findings were observed. On the day of dosing, animals were observed for clinical signs at 1-2 hours after dosing only.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable but with restrictions (reliable with restrictions) due to the deviations noted above.

Executive Summary
The test substance, glyphosate acid technical, was evaluated for its acute oral toxicity potential using 5 female and 5 male rats. The test substance was administered by oral gave in a single dose at 2000 mg/kg bw. No clinical signs or mortality were recorded during the study period of 14 days. The gross necropsy conducted at termination of the study revealed slightly congested lungs, splenomegaly and centrilobular hepatic congestion in male and female animals. The acute oral LD_{50} was determined to be: LD_{50}, oral, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate acid

Identification:	Glyphosate acid technical
Description:	Fine white granular powder
Lot/Batch #:	1073
Purity:	97.6% (certificate of analysis)
Stability of test compound:	Expiry date: Aug 1996

2. Vehicle and/or positive control: Cotton seed oil

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at receipt:	150.2 – 237.2 g (males) and 205.6 – 260.9 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Pelleted feed, *ad libitum*, except for a fasting period the night before dosing (approximately 14 – 18 h) and 3 - 4 hours after dosing
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C Humidity: 62 – 73% Air changes: Not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Feb 1995 – 13 Mar 1995
Finalisation date: 08/08/1995

Animal assignment and treatment:
Glyphosate acid was tested for acute oral toxicity using 5 males and 5 female rats. Prior to dosing, all animals were fasted overnight (approximately 14 – 18 h). Glyphosate acid was administered as a single oral dose by gavage at a concentration of 2000 mg/kg bw. A dose volume of 20 mL per kg body weight was not exceeded.

After dosing, the animals were fasted 3 – 4 hours. Individual body weights were measured prior to dosing. The animals were observed for clinical signs and mortality 1 – 2 hours after treatment and daily thereafter for the rest of the study period of 14 days. All animals were sacrificed at the end of the study period and subjected to a gross pathological examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed in any of the animals.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

The gross necropsy conducted at termination of the study revealed slightly congested lungs, splenomegaly and centrilobular hepatic congestion in male and female animals.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate acid technical in male and female rats was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in rat is above 2000 mg/kg bw.
According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
The acute oral LD_{50} of glyphosate acid technical in male and female rats is above 2000 mg/kg bw. This is in line with the conclusion from the initial evaluation (DAR, 1998). In contrast to the applicant's conclusion, the study is only considered acceptable (reliable with restrictions) due to the noted deviations.

B.6.2.1.18. *Study 18*

Data point:	CA 5.2.1/018
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD50 tests with [redacted] Glyphosate 62 % IPA in rats, limit test"
Report No	00926
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), EPA Pesticide Assessment Guidelines, Subdivison F, Series 81-1 (1984)
Deviations from current test guideline (OECD 420, 2001)	5 animals of both sexes were used. Air changes not specified. Body weights only recorded once prior to start of study and not once a week during the study. Individual body weights were not reported. Individual clinical signs were not reported, however it was mentioned that no clinical findings were observed. On the day of dosing, animals were observed for clinical signs at 1-2 hours after dosing only.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered to be acceptable (reliable with restrictions) due to the deviations noted above.

Executive Summary
The test substance, glyphosate salt, was evaluated for its acute oral toxicity potential using 5 female and 5 male rats. The test substance was administered by oral gave in a single dose at 2000 mg/kg bw. No clinical signs or mortality were recorded during the study period of 14 days. The gross necropsy conducted at termination of the study revealed severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion and subcapsular renal petechiae in all male and female animals. The acute oral LD_{50} was calculated to be: LD_{50}, oral, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	[redacted] Glyphosate 62 % IPA
Description:	Light greenish viscous liquid
Lot/Batch #:	940950
Purity:	62 % (certificate of analysis)
Stability of test compound:	Aug 1996 (expiry date)

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified (young adult)
Sex:	Male and female
Weight at receipt:	153 – 212.2 g (males) and 197.2 – 248.7 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Pelleted feed, *ad libitum*, except for a fasting period overnight (approximately 14 to 18 hours) before dosing and 3 - 4 hours after dosing
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C Humidity: 62 – 73 % Air changes: Not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 23 Feb 1995 – 13 Mar 1995
Finalisation date: 08/08/1995

Animal assignment and treatment:
Glyphosate salt was tested for oral toxicity using 5 male and 5 female rats. Prior to dosing, all animals were fasted overnight (approximately 14 – 18 hours). Glyphosate salt was administered as a single oral dose by

gavage at a concentration of 2000 mg/kg bw and a dose volume of 20 mL per kg body weight was not exceeded. After dosing, the animals were fasted 3 – 4 hours. Individual body weights were measured prior to dosing. The animals were observed for clinical signs and mortality 1 – 2 hours after treatment and daily thereafter for the rest of the study period of 14 days. All animals were sacrificed at the end of the study period and subjected to a gross pathological examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were observed in any of the animals.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

The gross necropsy conducted at termination of the study revealed severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion and subcapsular renal petechiae in all male and female animals (see table below).

Table B.6.2.1.18-1: Final report for "Oral and dermal LD50 tests with [redacted] Glyphosate 62 % IPA in rats, limit test" [redacted] 1995): Findings at gross necropsy

Dose level	**2000 mg/kg bw**	
Sex	**Males**	**Females**
Findings		
Severe lung congestion	5/5	5/5
Splenomegaly	5/5	5/5
Hepatomegaly with centrilobular congestion	5/5	5/5
Subcapsular renal petechiae	5/5	5/5

III. CONCLUSIONS

The acute oral LD_{50} of the glyphosate salt in male and female rats was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance to the former OECD guideline 401 (1987). Therefore, the outcome can be reported as valid. Minor deviations such as number of air changes or the use of more animals than requested, did not affect the study outcome. The study is however only considered supportive. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
The acute oral LD_{50} of glyphosate IPA salt in male and female rats is above 2000 mg/kg bw. This is in line with the conclusion from the initial evaluation (DAR, 1998). The study is only considered acceptable (reliable with restrictions) due to the noted deviations.

B.6.2.1.19. *Study 19*

Data point:	CA 5.2.1/019
Report author	[redacted]
Report year	1995

Report title	Acute oral toxicity of glyphosate technical 95% in the rat
Report No	10670
Document No	Not reported
Guidelines followed in study	US EPA Subdivision F, 81-1
GLP	Yes
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of T1586.3 Glyphosate Technical 95% was investigated in male and female rats (Sprague-Dawley, 6-8 weeks, 116-186 g at dosing, 5 animals/sex/dose). The vehicle used for dosing was CMC. A dose ranging study in pairs of rats indicated that the oral LD_{50} value was greater than 5000 mg/kg bw. Accordingly, the dose level in the main study was selected as 5000 mg/kg bw. Administration occurred orally by means of a gavage at a volume of 10 mL/kg bw. Rats were weighed prior to dosing and at 7 and 14 days after dosing. Clinical signs were recorded for 14 days following dosing before rats were sacrificed. Afterwards, all rats were subjected to necroscopy.
Short description of results:	No deaths occurred and clinical signs included piloerection, subdued behaviour and hunched appearance (2 hours to 1 day after dosing). Body weight gain was normal. No abnormalities were detected at necroscopy. The LD_{50} for T1586.3 Glyphosate Technical 95% was determined to be greater than 5000 mg/kg bw in male and female rats.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, Category 4a **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. Although the study was not explicitly performed according to OECD guidelines, the study is in accordance with guidelines for acute oral toxicity testing. The study is considered valid. The LD_{50} for T1586.3 Glyphosate Technical 95% was determined to be greater than 5000 mg/kg bw in male and female rats. This in in line with the conclusion from the first evaluation (DAR, 1998).
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** See above.

B.6.2.1.20. *Study 20*

Data point:	CA 5.2.1/020
Report author	[redacted]
Report year	1994
Report title	Glyphosate Premix: Acute Oral Toxicity (Limit Test) in the Rat
Report No	545/37
Document No	Not reported
Guidelines followed in study	EPA OTS 798.1175 (Acute Oral Toxicity) EPA OPP 81-1 (Acute Oral Toxicity)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. At dosing, animals were 5 to 8 weeks instead of 8 to 12 weeks old. Animals in range-

	finding study were observed for 5 days instead for at least 14 days. These deviations, however, are not expected to significantly impact the study outcome.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

Executive Summary
The acute oral toxicity of glyphosate premix was investigated in male and female rats. Therefore, a dose-range finding study with 1 animal per sex was performed. The animals were dosed once by oral gavage at a concentration of 5000 mg/kg bw. As no signs of toxicity were observed during the 5-day observation time following dosing, the main study was conducted. Therefore, 5 further animals of each sex were administered the test substance by oral gavage once at a concentration of 5000 mg/kg bw and a dose volume of 4.1 mL/kg bw. The animals were regularly observed for clinical signs after 0.5, 1, 2, and 4 hours after dosing and once daily thereafter for 14 days. Body weights were determined immediately prior to dosing, at day 7 and day 14. At the end of the observation period, all animals were sacrificed and subjected to gross necropsy.
No adverse clinical signs were noticed. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted.
The acute oral LD_{50} was determined to be: LD_{50}, oral, rats > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Premix
Description:	Pale yellow liquid, aqueous solution containing the isopropylamine salt of glyphosate as the active ingredient
Lot/Batch #:	290-JaK-146-4
Purity:	46.1% (Glyphosate), 62.2% (Glyphosate isopropylamine salt)
Stability of test compound:	30 September 1995 (expiry date)

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	5 – 8 weeks
Sex:	Males and females
Weight at dosing:	128 – 155 g (males), 125 – 137 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Rat and mouse expanded diet no. 1, *ad libitum* (Except for a fasting period the night prior to dosing and 2 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex in solid-floor polypropylene cages with woodflakes

Environmental conditions: Temperature: 19 – 22 °C
Humidity: 42 – 55%
Air changes: 15 times / hour
12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 28/03/1994 to 26/04/1994 (Duration of the study- not further specified)

Animal assignment and treatment:
Rats were housed by sex (5 males and 5 females) and starved the night prior to dosing. The undiluted test material was administered by a single oral gavage at dose level of 5000 mg/kg bw with an application volume of 4.1 mL/kg bw. Animals were starved for a further 2 hours following dosing. All animals were observed for clinical signs of toxicity at several time points on the day of administration (0.5, 1, 2 and 4 hours) and at least once a day, thereafter. Body weights were recorded prior to administration, on day 7 and prior to sacrifice on day 14. At study termination all animals were sacrificed followed by gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the range-finding or main study.

B. CLINICAL OBSERVATIONS

No adverse observations were made in the range-finding or main study.

C. BODY WEIGHT
Body weight gain of all animals was within the normal range (see table below).

Table B.6.2.1.20-1: Glyphosate Premix: Acute Oral Toxicity (Limit Test) in the Rat [redacted] 1994): Body weight data of the main study

Dose level	**5000 mg/kg bw**									
Sex	**Males**			**Females**			**Males**		**Females**	
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0-7**	**0-14**	**0-7**	**0-14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**			
3-0 (4-0)	155	205	253	129	165	185	50	48	36	20
3-1 (4-1)	154	210	250	125	158	174	56	40	33	16
3-2 (4-2)	128	158	184	137	174	202	30	26	37	28
3-3 (4-3)	140	196	243	127	160	175	56	47	33	15
3-4 (4-4)	137	198	255	132	168	186	61	57	36	18
Mean ± SD	**142.8 ± 11.6**	**193.4 ± 20.6**	**237.0 ± 30.0**	**130.0 ± 4.7**	**165.0 ± 6.4**	**184.4 ± 11.3**	**50.6 ± 12.2**	**43.6 ± 11.5**	**35.0 ± 1.9**	**19.4 ± 5.2**

§ = animal No. of males and (females)
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY
No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The acute oral LD_{50} of the test material (glyphosate premix) in male and female rats was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:

The study was performed according to a national guidelines similar to the current OECD guideline 420 (2001). Nevertheless, due to deviations and the composition of the test material the study is considered as supplementary information. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
The acute oral LD_{50} of glyphosate premix in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the initial evaluation (DAR, 1998). In contrast to the applicant's conclusion, the study is considered acceptable since it is not expected that the noted deviations have a significant impact on the study outcome.

B.6.2.1.21. *Study 21*

Data point:	CA 5.2.1/021
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute oral toxicity (limit test) in the rat
Report No	710/14
Document No	Not reported
Guidelines followed in study	OECD 401 (1987) Method B1 of Commission Directive 92/69/EEC
GLP	Yes
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of Glyphosate Technical 95% was investigated in male and female rats (Sprague-Dawley, 5-8 weeks, 140-165 g (males) and 154-165 g (females) at dosing, 5 animals/sex/dose). The vehicle used for dosing was arachis oil B.P. Following a range-finding study, the dose level in the main study was selected as 2000 mg/kg bw. Administration occurred orally by means of a gavage at a volume of 10 mL/kg bw. Rats were weighed prior to dosing and at 7 and 14 days after dosing. Clinical signs were recorded for 14 days following dosing before rats were sacrificed. Afterwards, all rats were subjected to necroscopy.
Short description of results:	No deaths occurred and no clinical signs were noted. Body weight gain was normal. No abnormalities were detected at necroscopy. The LD_{50} for Glyphosate Technical 95% was determined to be greater than 2000 mg/kg bw in male and female rats.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4a* **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered valid. The LD_{50} for Glyphosate Technical 95% was determined to be greater than 2000 mg/kg bw in male and female rats. This in in line with the conclusion from the first evaluation (DAR, 1998).
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL. **Conclusion AGG:** See above.

B.6.2.1.22. *Study 22*

Data point:	CA 5.2.1/022
Report author	[redacted]
Report year	1994
Report title	Acute oral toxicity in rats
Report No	[redacted]-94-401/R
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Five animals of both sexes per group used. Control group included. Organ weights measured. No significant impact on the study outcome expected.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

Executive Summary
The test substance, glyphosate technical was evaluated for its acute oral toxicity potential. 5 female and 5 male rats were administered glyphosate technical (in 3 % carboxymethyl-cellulose in water) by oral gavage in a single dose at 5000 mg/kg bw. A control group was included with 5 male and 5 female animals. No clinical signs or mortality were recorded during the study period of 14 days. Body weights of treated male animals were statistically significantly lower at the end of the study period. The gross necropsy conducted at termination of the study did not reveal any abnormalities. Organ weights were comparable to the control, except for male heart weight, which was statistically significantly decreased but not considered to be of toxicological relevance. The acute oral LD_{50} was determined to be: LD_{50}, oral, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Technical
Description: White or almost white crystalline powder
Lot/Batch #: 36300892
Purity: 97.2% (certificate of analysis)
Stability of test compound: Expiry date: Sep 1994

2. Vehicle and/ or positive control: 3 % carboxymethyl-cellulose in water

3. Test animals:

Species: Rat
Strain: Wistar
Source: [redacted]
Age: Not specified
Sex: Male and female
Weight at receipt: Not specified
Acclimation period: Approximately 3 weeks

Diet/Food:	Altromin rodent chow, *ad libitum*, except for a fasting period of 16 hours before dosing and 4 hours after dosing
Water:	Tap water, *ad libitum*
Housing:	Groups of 5/sex in marolon III. box
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 45 – 70% Air changes: 10 times/h 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 17 Jan 1994 – 31 Jan 1994
Finalisation date: 06 Apr 1994

Animal assignment and treatment:
Glyphosate technical was tested for acute oral toxicity using 5 males and 5 female rats per dose group. 16 hours prior to dosing, all animals were fasted. Glyphosate technical was administered as a single oral dose by gavage at a concentration of 5000 mg/kg bw. A control group was included as well. After dosing, the animals were fasted 4 hours. Individual body weights were measured prior to dosing and weekly thereafter. The animals were observed for clinical signs and mortality on the day of dosing and twice a day thereafter for the rest of the study period of 14 days. All animals were sacrificed at the end of the study period and subjected to a gross pathological examination. Furthermore, organ weights of the brain, heart, thymus, stomach, spleen, lung, liver, kidneys and adrenals were measured of both sexes. In addition, organ weights of testes and epididymides were measured of male animals. The ratio of organ to brain weight was calculated.

Statistical analysis:
The data were analysed for statistical significance using the t-test. A p-value of <0.05 indicated statistical significance.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in any animal of the control or treated group.

C. BODY WEIGHT
No differences in body weight or body weight gain were observed in female rats when compared to the control group. In males however, a statistically significant decrease in body weight gain (-14.9%) was observed in the treated group during the second week (see table below).

Table B.6.2.1.22-1: Acute oral toxicity in rats [redacted] 1994): Summary of body weights recorded during the study in control and treated animals.

Day		**0**	**7**	**14**	**0-14**	**0**	**7**	**14**	**0-14**
Sex		**Males**				**Females**			
Dose level (mg/kg bw)	**Animal No.§**	**Body weight [g]**			**Bw gain [g]**	**Body weight [g]**			**Bw gain [g]**
0	6 (16)	205	250	285	80	135	160	175	40
	7 (17)	210	270	315	105	150	175	195	45
	8 (18)	200	260	300	100	160	190	205	45
	9 (19)	190	255	280	90	145	170	175	30
	10 (20)	195	250	290	95	145	165	175	30
	Mean	**200.0**	**257.0**	**294.0**	**94.0**	**147.0**	**172.0**	**185.0**	**38.0**

Table B.6.2.1.22-1: Acute oral toxicity in rats [redacted] 1994): Summary of body weights recorded during the study in control and treated animals.

Day		**0**	**7**	**14**	**0-14**	**0**	**7**	**14**	**0-14**
Sex		**Males**				**Females**			
Dose level (mg/kg bw)	**Animal No.§**	**Body weight [g]**			**Bw gain [g]**	**Body weight [g]**			**Bw gain [g]**
	± SD	**± 7.9**	**± 8.4**	**± 13.9**	**± 9.6**	**± 9.1**	**± 11.5**	**± 14.1**	**± 7.6**
5000	1 (11)	195	240	270	75	150	170	180	30
	2 (12)	205	250	275	70	160	185	195	35
	3 (13)	190	245	275	85	145	165	180	35
	4 (14)	200	250	280	80	145	170	185	40
	5 (15)	205	260	295	90	145	165	185	40
	Mean	**199.0**	**249.0**	**279.0**	**80.0***	**149.0**	**171.0**	**185.0**	**36.0**
	± SD	**± 6.5**	**± 7.4**	**± 9.6**	**± 7.9**	**± 6.5**	**± 8.2**	**± 6.1**	**± 4.2**

§ = animal No. of males and (females), * $p<0.05$

D. ORGAN WEIGHTS

The absolute organ weights were similar to the controls. The heart weight was significantly lower in male rats at 5000 mg/kg bw. However, this finding is not considered to be of toxicological relevance (see table below). However, the RMS considers that since no histopathological evaluation was conducted toxicological relevance of the decreased heart weight cannot be fully excluded.

Table B.6.2.1.22-2: Acute oral toxicity in rats [redacted] 1994): Summary of absolute organ weights and organ to brain weight ratios.

Dose group (mg/kg bw)		**0**	**5000**	**0**	**5000**	**0**	**5000**	**0**	**5000**
Sex		**Males**		**Females**		**Males**		**Females**	
Absolute organ weight [g]						**Relative organ weight [g]**			
Brain	Mean	1.89	1.88	1.66	1.68	1.89	1.88	1.66	1.68
	± SD	± 0.07	± 0.10	± 0.07	± 0.09	± 0.07	± 0.10	± 0.07	± 0.09
Heart	Mean	0.92	**0.84**	0.64	0.65	0.49	0.45	0.39	0.39
	± SD	± 0.07	**± 0.04***	± 0.10	± 0.06	± 0.04	± 0.04	± 0.07	± 0.05
Thymus	Mean	0.66	0.58	0.46	0.44	0.35	0.31	0.28	0.27
	± SD	± 0.11	± 0.04	± 0.12	± 0.07	± 0.06	± 0.03	± 0.07	± 0.04
Stomach	Mean	1.53	1.54	1.19	1.19	0.81	0.82	0.72	0.71
	± SD	± 0.13	± 0.14	± 0.12	± 0.09	± 0.05	± 0.10	± 0.09	± 0.07
Spleen	Mean	0.70	0.65	0.51	0.49	0.37	0.35	0.31	0.29
	± SD	± 0.11	± 0.05	± 0.04	± 0.07	± 0.04	± 0.03	± 0.03	± 0.05
Lung	Mean	1.69	1.63	1.29	1.35	0.90	0.87	0.78	0.81
	± SD	± 0.19	± 0.14	± 0.11	± 0.06	± 0.10	± 0.09	± 0.09	± 0.08
Liver	Mean	13.33	13.00	8.27	8.34	7.05	6.93	5.00	4.99
	± SD	± 0.86	± 1.00	± 1.25	± 0.87	± 0.27	± 0.69	± 0.83	± 0.69
Kidneys	Mean	2.12	2.06	1.29	1.29	1.12	1.10	0.78	0.77
	± SD	± 0.23	± 0.18	± 0.20	± 0.09	± 0.09	± 0.11	± 0.13	± 0.05
Adrenals	Mean	0.05	0.05	0.06	0.07	0.03	0.03	0.04	0.04
	± SD	± 0.01	± 0.00	± 0.02	± 0.01	± 0.00	± 0.00	± 0.01	± 0.01
Testes	Mean	3.17	2.98			1.68	1.58		
	± SD	± 0.17	± 0.20			± 0.10	± 0.10		
Epididymis	Mean	0.75	0.66			0.40	0.35		
	± SD	± 0.02	± 0.08			± 0.02	± 0.03		

*$p<0.05$

E. NECROPSY

No abnormal findings were recorded during necropsy.

III. CONCLUSIONS

Based on the results the test substance glyphosate technical is considered as non-toxic concerning oral toxicity.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in rat is above 5000 mg/kg bw as no mortality occurred during the study. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
In it agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate technical in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the initial evaluation (DAR, 1998).

B.6.2.1.23. *Study 23*

Data point:	CA 5.2.1/023
Report author	[redacted]
Report year	1994
Report title	Glyphosate technical: Acute oral toxicity study in mice
Report No	940020
Document No	Not reported
Guidelines followed in study	OECD 401, EEC B.1
GLP	Yes
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of Glyphosate Technical (purity not reported) was investigated in male and female mice (Crl:CD-1 (ICR), 34-40 days at dosing, 22-25 g at receipt, 5 animals/sex/dose). The vehicle used for dosing was 0.5% methylcellulose in water. Following a range-finding study, the dose level in the main study was selected as 2000 mg/kg bw. Administration occurred orally by means of a gavage at a volume of 20 mL/kg bw. Mice were weighed prior to dosing and at 3, 8 and 14 days after dosing. Clinical signs were recorded for 14 days following dosing before rats were sacrificed. Afterwards, all rats were subjected to necroscopy.
Short description of results:	No deaths occurred. Clinical findings were piloerection, hunched posture, and hypoactivity (2 hours tom 2 days after dosing). Body weight gain was normal. No abnormalities were detected at necroscopy. The LD_{50} for Glyphosate Technical was determined to be greater than 2000 mg/kg bw in male and female mice.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000; Category 4a **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered to be valid. The LD_{50} for Glyphosate Technical was determined to be greater than 2000 mg/kg bw in male and female mice. This in in line with the conclusion from the first evaluation (DAR, 1998).
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have

	to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** See above.

B.6.2.1.24. *Study 24*

Data point:	CA 5.2.1/024
Report author	[redacted]
Report year	1992
Report title	Glyphosate technical Acute oral toxicity (Limit test) in the rat
Report No	134/37
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	6 animals per sex used instead of 5 in total. Animals were 5 – 8 weeks old instead of 8 – 12 weeks. No significant impact on the study outcome expected.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable

Executive Summary
The acute oral toxicity of glyphosate was investigated in 5 male and 5 female rats of the Sprague-Dawley strain. The test substance was administered undiluted by oral gavage to each animal at a dosage of 2000 mg/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study. No mortality occurred. No clinical signs were noticed. Changes of body weight were in the normal range. No abnormalities at necropsy were observed. The acute oral LD50 was determined to be: LD50, oral, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate technical
Description: White powder
Lot/Batch #: L3258
Purity: Not specified
Stability of test compound: Not specified

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species: Rat
Strain: Sprague-Dawley
Source: [redacted]
Age: 5 – 8 weeks
Sex: Male and female

Weight at dosing:	142 – 150 g (males), 129 – 142 g (females)
Acclimation period:	At least 5 days
Diet/Food:	Rat and mouse extended Diet No. 1 (SDS Limited), *ad libitum* (except for fasting period overnight before dosing)
Water:	Not specified, *ad libitum*
Housing:	Five per sex in solid-floor polypropylene cages with sawdust bedding.
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 52 – 63% Air changes: 15 per hour light/dark cycle: 12 h / 12 h

B. STUDY DESIGN AND METHODS

In life dates: 11/12/1991 to 20/01/1992

Animal assignment and treatment:
A group of 5 male and 5 female rats was fasted overnight and dosed with the test substance at a concentration of 2000 mg/kg bw and a dose volume of 10 mL/kg bw. After treatment, animals were observed for mortality and clinical signs four times within the first 4 hours after dosing and once daily thereafter. Surviving animals were sacrificed after 14 days and were subjected to necropsy. Body weights were recorded immediately before dosing and on day 7 and 14 during the study.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14 days observation period.

C. BODY WEIGHT
All animals gained weight during the study (see table below).

Table B.6.2.1.24-1: Acute oral toxicity of Glyphosate [redacted] 1992): Mean body weight

Dose group	**2000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**14**	**0**	**7**	**14**
Animal No.§	**Body weight [g]**					
281 (286)	146	209	272	141	177	206
282 (287)	142	213	275	138	182	219
283 (288)	150	220	294	129	170	197
284 (289)	150	210	279	133	173	209
285 (290)	149	210	277	142	174	209
Mean (± SD)	**147.4 ± 3.4**	**212.4 ± 4.5**	**279.4 ± 8.6**	**136.6 ± 5.5**	**165.2 ± 23.5**	**208.0 ± 7.9**

§ = animal No. of males and (females)
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively

D. NECROPSY
No abnormalities were observed during necropsy.

III. CONCLUSIONS

The acute oral LD_{50} of the test material in male and female rats was greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in concordance to the former OECD guideline 401 (1987). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate technical in male and female rats is above 2000 mg/kg bw. This is in line with the conclusion from the first evaluation (DAR, 1998).

B.6.2.1.25. *Study 25*

Data point:	CA 5.2.1/025
Report author	[redacted]
Report year	1991
Report title	Assessment of acute oral toxicity of "Glyphosate technical" to mice
Report No	12321
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes used instead of one sex (usually females). Animals were only 4-5 weeks old at the beginning of the study. Fasting periods in mice prior to dosing 18 hours instead of 3-4 hours and 4 hours instead of 1-2 hours after dosing. No observation 30 minutes after dosage. Individual weights are not reported. No significant impact on the study outcome is expected.
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 to be acceptable.

Executive Summary
The acute oral toxicity of glyphosate technical was investigated in male and female mice (5 animals/sex/group) of the Bom:NMRI strain. The test substance was administered once by oral gavage to the starved animals at a dosage of 2000 mg/kg bw and constant dose volume of 10 mL/kg bw. The animals were observed 1, 3 and 6 hours after dosing. Furthermore, mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study. No adverse clinical signs were noticed. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted. The acute oral LD50 was calculated to be: LD50, oral, mice > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate
   - Identification: Glyphosate Technical (PMG)
   - Description: White powder
   - Lot/Batch #: 206-JaK-25-1
   - Purity: 98.6%
   - Stability of test compound: Not specified
2. **Vehicle and/ or positive control:** Distilled water

3. Test animals:

Species:	Mice
Strain:	Bom:NMRI
Source:	[redacted]
Age:	4 – 5 weeks
Sex:	Male and female
Weight at dosing:	21 – 24 g
Acclimation period:	5 days
Diet/Food:	Complete rodent diet "Altromin 1314", *ad libitum* (Except for a fasting period of 18 hours prior to dosing and 4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex in Macrolone cages Type II (26 × 20 × 14 cm) Pinewood sawdust "Spanvall Special White" bedding
Environmental conditions:	Temperature: 21 ± 3 °C Humidity: 55 ± 15% Air changes: 6 times / hour 12-hour light / dark cycle (light during 06:00 – 18:00)

B. STUDY DESIGN AND METHODS

In life dates: No information

Animal assignment and treatment:
Mice were housed by sex (5 males and 5 females) and starved for approximately 18 hours prior to dosing. Test material was suspended in distilled water and administered by a single oral gavage at dose level of 2000 mg/kg bw with an application volume of 10 mL/kg bw. Animals were starved for a further 3-4 hours following dosing. All animals were observed for clinical signs of toxicity at several time points on the day of administration and at least once a day, thereafter. Body weights were recorded prior to administration, on day 7 and prior to sacrifice on day 14. At study termination all animals were sacrificed with carbon dioxide followed by gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-days observation period.

B. CLINICAL OBSERVATIONS

1 and 3 hours after treatment piloerection and sedation were observed. Piloerection was observed up to 6 hours after treatment.

Table B.6.2.1.25-1: Assessment of acute oral toxicity of "Glyphosate technical" to mice [redacted] 1991): Summary of clinical observations

Dose group	**2000 mg/kg bw**							
Sex	**Males**				**Females**			
Time after treatment	1 h	3 h	6 h	Day 1 - 14	1 h	3 h	6 h	Day 1 - 14
Total animals n examined	5	5	5	5	5	5	5	5
Clinical sign n	5	5	5	0	5	5	5	0
Piloerection n	5	5	5	0	5	5	5	0
Sedation n	5	5	0	0	5	5	0	0
Single animals observations								
Animal No.§								

Table B.6.2.1.25-1: Assessment of acute oral toxicity of "Glyphosate technical" to mice (1991): Summary of clinical observations

Dose group	**2000 mg/kg bw**							
Sex	**Males**				**Females**			
Time after treatment	1 h	3 h	6 h	Day 1 - 14	1 h	3 h	6 h	Day 1 - 14
6 (1)	AB	AB	A	*	AB	AB	A	*
7 (2)	AB	AB	A	*	AB	AB	A	*
8 (3)	AB	AB	A	*	AB	AB	A	*
9 (4)	AB	AB	A	*	AB	AB	A	*
10 (5)	AB	AB	A	*	AB	AB	A	*

§ = animal No. of males and (females), * = No abnormalities detected, A = piloerection, B = sedation

C. BODY WEIGHT

Body weight gain of all animals was within the normal range. A summary of the body weights is provided in the table below.

Table B.6.2.1.25-2: Assessment of acute oral toxicity of "Glyphosate technical" to mice 1991): Body weight data

Dose level	**2000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**14**	**0**	**7**	**14**
Body weight [g]						
Mean ± SD	21.6 ± 0.5	32.0 ± 1.7	36.8 ± 2.3	23.8 ± 0.4	25.6 ± 0.5	28.0 ± 1.2

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The acute oral LD_{50} of glyphosate in mice was estimated to be greater than 2000 mg/kg bw.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is obsolete nowadays. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in mice is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate technical (PMG) in male and female mice is above 2000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.26. *Study 26*

Data point:	CA 5.2.1/026
Report author	
Report year	1991
Report title	Acute oral toxicity study with glyphosate technical (FSG 0309 H/05 March 90) in Wistar rats
Report No	.874.AOR
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)

Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group, no stepwise procedure; highest dose group above exceptionally considered 5000 mg/kg bw; maximum dose volume was exceeded at the highest dose level (25 mL/kg bw instead of 20 mL/kg bw). Day of clinical observations not provided.
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 to be acceptable (reliable with restrictions) due to the deviations noted above.

Executive Summary
The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential. 5 female and 5 male rats were used per dose group. The test substance was administered by oral gavage at levels of 2500, 5000 and 7500 mg/kg bw. No mortality and no clinical signs occurred at the two low dose groups. At the high dose level of 7500 mg/kg bw, 4/10 animals died and clinical signs included lethargy, ataxia and dyspnoea. Body weight decreased in animals that died during the study. Some surviving rats lost weight at day 7, however, gained weight until end of the study, except for 2 rats which lost weight up to the end of the observation period. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, rat > 7500 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical
Description:	Solid white crystals
Lot/Batch #:	60
Purity:	96.80%
Stability of test compound:	Expiry date July 1992

2. Vehicle and/or positive control: Refined groundnut (peanut) oil

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	11 weeks
Sex:	Male and female
Weight at dosing:	140 – 196 g (males), 122 – 210 g (females)
Acclimation period:	At least one week
Diet/Food:	Standard 'Gold Mohur' brand pelleted rat feed, *ad libitum* except for approx. 14 – 16 h before and 3 h after dosing
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Groups of 5/sex in standard polypropylene cages with stainless steel top grill. Bedding: steam sterilised clean paddy husk.
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 68 ± 6% Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No information given

Animal assignment and treatment:
Groups of 5 fasted females and 5 males were assigned to three different dose groups. The animals received the test substance at dose levels of 2500, 5000 and 7500 mg/kg bw by oral gavage. The dosing volume was 8.3, 16.6 and 25.0 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made four times on the day of dosing (Day 0) and once daily thereafter for 14 days (Days 2 – 14). Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, surviving animals were sacrificed. All study animals (dead and surviving) were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities at 2500 and 5000 mg/kg bw. However, 40 % of the animals died at 7500 mg/kg bw (see table below).

Table B.6.2.1.26-1: Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Mortality for all dose groups

Dose level (mg/kg bw)	Animal No.		No. of deaths/No. of animals examined (Day of death)	
	Males	Females	Males	Females
2500	0441 – 0445	446 – 450	-	-
5000	0451 – 0455	456 – 460	-	-
7500	0461	0466	1/5 (day 3)	-
	0462	0467	-	1/5 (day 2)
	0463	0468	-	-
	0464	0469	1/5 (day 1)	1/5 (day 3)
	0465	0410	-	-

- = No death

B. CLINICAL OBSERVATIONS

Clinical signs were noted in animals of the high dose group (7500 mg/kg bw) and included lethargy, ataxia and dyspnoea. The table below shows individual clinical observations.

Table B.6.2.1.26-2: Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Individual clinical observations

Dose level (mg/kg bw)	Animal No.		Clinical observations	
	Males	Females	Males	Females
2500	0441 – 0445	446 – 450	-	-
5000	0451 – 0455	456 – 460	-	-
7500	0461	0466	-	-
	0462	0467	-	Lethargy, ataxia and dyspnoea
	0463	0468	-	-
	0464	0469	-	Lethargy
	0465	0470	-	Lethargy

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance at 2500 and 5000 mg/kg bw, except for a few animals that lost weight on day 7 but exceeded their initial weight on day 14. Two female animals in the mid dose group lost weight by Day 14 compared to their initial weight. Dead animals from the high dose group had lost weight. Individual body weights are provided in the table below.

Table B.6.2.1.26-3: Acute oral toxicity study with glyphosate technical (FSG 03090 h/05 March 90) in Wistar rats ([redacted] 1991): Body weight development

Day		**0**	**7**	**14**	**0**	**7**	**14**
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.**	**Body weight [g]**					
2500	0441 (0446)	172	172	174	126	152	152
	0442 (0447)	140	156	156	134	136	162
	0443 (0448)	186	202	204	180	174	220
	0444 (0449)	186	204	218	122	112	150
	0445 (0450)	148	166	166	180	194	196
	Mean	**166.4**	**180**	**183.6**	**148.4**	**153.6**	**176**
	± SD	**±21.4**	**± 21.8**	**± 26.3**	**± 29.2**	**± 32.0**	**± 30.8**
5000	0451 (0456)	170	162	210	154	172	190
	0452 (0457)	176	192	208	168	140	150
	0453 (0458)	170	166	202	156	118	154
	0454 (0459)	194	204	234	148	154	166
	0455 (0460)	142	144	144	172	188	196
	Mean	**170.4**	**173.6**	**199.6**	**159.6**	**154.4**	**171.2**
	± SD	**± 18.7**	**± 24.1**	**± 33.4**	**± 10.0**	**± 27.3**	**± 20.9**
7500	0461 (0466)	196	-	-(176)[#]	210	230	258
	0462 (0467)	186	202	222	184	-	-(164)[#]
	0463 (0468)	174	184	184	184	152	194
	0464 (0469)	190	-	-(174)[#]	150	-	-(126)[#]
	0465 (0470)	176	182	206	152	174	174
	Mean	**184.4**	**189.3**	**204.0**	**176**	**191.0**	**208.7**
	± SD	**± 9.3**	**± 11.0**	**± 19.1**	**± 25.2**	**± 40.2**	**± 43.9**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.
Body weight of decedents, not used for calculation of the mean and the SD.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities in dead and surviving animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female rats was determined to be greater than 7500 mg/kg bw.

Assessment and conclusion by applicant:

The study is performed according to OECD guideline 401, which is obsolete nowadays.
Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 7500 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

It is agreed with the applicant's conclusion. The acute oral LD_{50} of Glyphosate Technical in male and female rats is above 7500 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015). It is noted, however, that the study is considered acceptable (reliable with restrictions) due to the noted deviations.

B.6.2.1.27. *Study 27*

Data point:	CA 5.2.1/027
Report author	[redacted]
Report year	1991
Report title	Acute oral toxicity study with glyphosate technical in swiss albino mice
Report No	[redacted].875.AOM
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes instead of one sex (usually females) with 5 animals per dose group. No stepwise procedure. Highest dose group above exceptionally considered 5000 mg/kg bw. Maximum dose volume was exceeded at the mid and high dose level as the test substance was administered in groundnut (peanut) oil. Animals 14 weeks old instead of 8 to 12 weeks. Day of clinical observations not provided.
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 to be acceptable (reliable with restrictions) due to the deviations noted above.

Executive Summary
The test substance, glyphosate, was evaluated for its acute oral toxicity potential. 5 female and 5 male mice were used per dose group. The test substance was administered by oral gavage at levels of 2500, 5000 and 7500 mg/kg bw. Mortality occurred in the respective dose groups as follows: 10 %, 20 %, and 40 %. Clinical signs included lethargy in all dose groups, ataxia and dyspnoea at 7500 mg/kg bw. Body weight decreased in animals that died during the study. A few surviving mice lost weight at day 7 however gained weight until end of the study, except for 2 mice which lost weight. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be: LD_{50}, oral, mice > 7500 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Technical
Description: Solid white crystals
Lot/Batch #: 60
Purity: 96.80%
Stability of test compound: Expiry date July 1992

2. Vehicle and/or positive control: Refined groundnut (peanut) oil

3. Test animals:

Species: Mice
Strain: Swiss albino
Source: [redacted]
Age: 14 weeks
Sex: Male and female
Weight at dosing: 24 – 40 g (males), 24 – 34 g (females)
Acclimation period: At least one week

Diet/Food:	Standard 'Gold Mohur' brand pelleted mice feed, *ad libitum* except for approx. 14 – 16 h before and 1 h after dosing
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays. Kept in glass bottles, *ad libitum*
Housing:	Groups of 5/sex in standard polypropylene cages with stainless steel top grill. Individual housing after dosing. Bedding: steam sterilised clean paddy husk.
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 68 ± 6% Air changes: 10 – 15 / hour 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No information given

Animal assignment and treatment:
Groups of 5 fasted females and 5 males were assigned to three different dose groups. The animals received the test substance at dose levels of 2500 (G1), 5000 (G2) and 7500 (G3) mg/kg bw by oral gavage. The dosing volume was 6.25, 12.5 and 18.75 mL/kg bw. Observations for mortality and clinical/behavioural signs of toxicity were made four times on the day of dosing (Day 0) and once daily thereafter for 14 days (Days 2-14). Individual body weights were recorded just prior to dosing and on Days 7 and 14. 14 days after dosing, each animal was euthanised using ether anaesthesia. All study animals (dead and surviving) were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality was observed between 1 and 8 days following treatment at dose levels of 2500 mg/kg bw (1/5 males, day 2), 5000 mg/kg bw (1/5 males, day 5 and 1/5 females, day 8), and 7500 mg/kg bw (3/5 males, days 1, 2) and 1/5 females, days 2). Details are provided in the table below.

Table B.6.2.1.27-1: Acute oral toxicity study with glyphosate technical in swiss albino mice [redacted] 1991): Mortality for all dose groups

Dose level (mg/kg bw)	Animal No.		Mortality*	
	Males	Females	Males	Females
2500	001 – 005	006 – 010	1 (004)[§] /5 (day 2)	0
5000	011 – 015	016 – 020	1 (013) /5 (day 5)	1 (020) /5 (day 8)
7500	021	026	1/5 (day 1)	-
	022	027	1/5 (day 2)	-
	023	028	-	-
	024	029	-	-
	025	030	1/5 (day 1)	1/5 (day 2)

* = No. of deaths/No. of animals examined (Day of death), - = No death, [§] = animal number

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity in decedents and survivors were observed in all treatment groups and included and included lethargy, urine incontinence, ataxia and dyspnoea. For detail see table below.

Table B.6.2.1.27-2: Acute oral toxicity study with glyphosate technical in swiss albino mice [redacted] 1991): Individual clinical observations

Dose level (mg/kg bw)	Animal No.		Clinical observations	
	Males	Females	Males	Females
2500	001 – 005	006 – 010	Lethargy (002, 003, 004)[§]	-
5000	011 – 015	016 – 020	Lethargy, urine incontinence (013)	Lethargy (020)

Table B.6.2.1.27-2: Acute oral toxicity study with glyphosate technical in swiss albino mice (1991): Individual clinical observations

7500	021	026	Lethargy	-
	022	027	Lethargy	-
	023	028	-	Lethargy
	024	029	Lethargy	-
	025	030	Lethargy, ataxia and dyspnoea	Lethargy

- = No observations made, §= animal number

C. BODY WEIGHT

Surviving animals with body weight loss or maintenance of body weight from days 1 to 7 gained or maintained weight during the 14-day observation period except for one female in the 5000 mg/kg bw group and one male in the 7500 mg/kg bw group. Individual body weights are provided in the table below.

Table B.6.2.1.27-3: Acute oral toxicity study with glyphosate technical in swiss albino mice 1991): Body weight data

Day		**0**	**7**	**14**	**0**	**7**	**14**
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.§**	**Body weight [g]**					
2500	001 (006)	24	24	26	34	34	34
	002 (007)	28	28	30	30	30	30
	003 (008)	32	34	32	32	32	- (32) #
	004 (009)	30	-	- (24)#	30	30	30
	005 (010)	34	32	34	30	32	32
	Mean	**29.6**	**29.5**	**30.5**	**31.2**	**31.6**	**31.5**
	± SD	**± 3.8**	**± 4.4**	**± 3.4**	**± 1.8**	**± 1.7**	**± 1.9**
5000	011 (016)	36	38	36	26	30	28
	012 (017)	40	40	40	30	26	30
	013 (018)	36	-	- (26) #	26	30	28
	014 (019)	34	36	36	24	32	26
	015 (020)	34	34	34	28	18	20
	Mean	**36**	**37.0**	**36.5**	**26.8**	**27.2**	**26.4**
	± SD	**± 2.4**	**± 2.6**	**± 2.5**	**± 2.3**	**± 5.6**	**± 3.8**
7500	021 (026)	36	-	- (32) #	28	34	34
	022 (027)	36	-	- (30) #	28	26	30
	023 (028)	36	30	34	24	22	24
	024 (029)	32	30	32	30	30	30
	025 (030)	34	-	- (30) #	26	-	- (22) #
	Mean	**34.8**	**30.0**	**33.0**	**27.2**	**28.0**	**29.5**
	± SD	**± 1.8**	**± 0.0**	**± 1.4**	**± 2.3**	**± 5.2**	**± 4.1**

§ = animal No. of males and (females), - = no determination of body weight, # body weight of decedents, not used for calculation of the mean and the SD.
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No necropsy findings in decedents and surviving animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female mice was greater than 7500 mg/kg bw.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is cancelled. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} in mice is above 7500 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of Glyphosate Technical in male and female mice are both above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015). It is noted, however, that the study is considered acceptable (reliable with restrictions) due to the noted deviations.

B.6.2.1.28. *Study 28*

Data point:	CA 5.2.1/028
Report author	[redacted]
Report year	1990
Report title	Acute oral toxicity study in the rat: Glyphosate technical
Report No	[redacted] 900823B
Document No	Not reported
Guidelines followed in study	OECD 401 (1987)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. Young animals were used, age not specified in detail. No fasting of further 3-4 hours after treatment. Clinical signs were not reported for individual animals. Number of air changes were not specified.
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 to be acceptable (reliable with restrictions) due to the deviations noted above.

Executive Summary
The acute oral toxicity of glyphosate technical was investigated in male and female rats (5 animals/sex/group) of the CD strain. The test substance, suspended in 1 % methylcellulose, was administered by oral gavage to each animal at a dosage of 0, 3000, 5000 and 8000 mg/kg bw at a constant dose volume of 20 mL/kg bw. Clinical signs were recorded at 0.5, 1 and 3 hours following dosing and daily thereafter for 14 days. All animals were subjected to a gross necropsy at the end of the study.
Clinical signs noticed were decreased activity, abnormal posture, abnormal limb position and abnormal gait in animals of the 5000 and 8000 mg/kg bw dose groups. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted. The acute oral LD50 was determined to be: LD50, oral, rat > 8000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Technical

Description: Yellowish (transparent)

Lot/Batch #: 0190 A

Purity:	98.1%
Stability of test compound:	Not specified

2. Vehicle and/or positive control: 1 % methylcellulose

3. Test animals:

Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Male and female
Weight at dosing:	184 – 229 g (males) 163 – 198 g (females)
Acclimation period:	5 days
Diet/Food:	Standard rat diet pellets (Redmills, Goresbridge, Co. Kilkenny Ireland), *ad libitum* (except the night prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	5 animals/sex/cage in flat bottomed polypropylene cages with stainless steel
Environmental conditions:	Temperature: 13 – 25 °C Humidity: 43 – 61% (Average) Air changes: Not specified 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 14/03/1990 to 28/03/1990 (experimental phase – without acclimation period)
Finalisation date: 23 August 1990

Animal assignment and treatment:
Rats were housed by sex (5 males and 5 females) and fasted overnight prior to dosing. The test material was suspended in 1 % methylcellulose and administered by a single oral gavage at dose levels of 3000, 5000 and 8000 mg/kg bw with an application volume of 20 mL/kg bw. A control group with 5 male and 5 female rats was included which received 1 % methylcellulose as a single oral gavage. All animals were observed for clinical signs of toxicity at 0.5, 1 and 3 hours post treatment and once a day thereafter for 14 days. Body weights were recorded prior to administration, on day seven and prior to sacrifice on Day 14. At study termination all animals were sacrificed followed by a gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-days observation period after administration.

B. CLINICAL OBSERVATIONS

Animals of the control and low dose group did not show any signs of toxicity. Decreased activity was observed in 5/5 males at 5000 mg/kg bw and 8000 mg/kg bw 20 min after dosing. However, activity seems normal after 1 hour, except for 1 animal at 5000 mg/kg bw. A further 2/5 females at 8000 mg/kg bw showed decreased activity 1 hour after dosing. At the next observation time point animals had recovered. 2/5 males at 5000 mg/kg bw showed abnormal gait 1 hour after treatment and one male had recovered at hour 3. 1/5 males showed abnormal posture between 1 and 3 hours after test substance administration. 1/5 females showed decreased activity but recovered by hour 3. Abnormal body posture and limb position was observed in 2/5 females at 8000 mg/kg bw after 1 hour and one of them showed abnormal gait at 1 and 3 hours of the observation period. Clinical signs are listed in the table below.

Table B.6.2.1.28-1: Acute oral toxicity study in the rat: Glyphosate technical [redacted] 1989): Clinical observations after treatment with glyphosate technical

Dose group [mg/kg bw]	Control		3000		5000		8000	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Total animals n examined	5	5	5	5	5	5	5	5
Clinical signs n	-	-	-	-	5	1	5	2
Decreased activity	-	-	-	-	5	1	5	2
Abnormal body posture	-	-	-	-	1	-	-	2
Abnormal gait	-	-	-	-	2	-	-	1
Abnormal limb position	-	-	-	-	-	-	-	1

C. BODY WEIGHT

Weight gain of all animals was within the normal range. For details on individual and mean body weights see table below.

Table B.6.2.1.28-2: Acute oral toxicity study in the rat: Glyphosate technical [redacted] 1989): Body weight development after treatment with glyphosate technical

Day		**0**	**7**	**14**	**0**	**7**	**14**
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.§**	**Body weight [g]**					
0	31 (36)	212	275	304	198	243	258
	32 (37)	211	302	332	189	233	240
	33 (38)	222	306	333	163	183	201
	34 (39)	184	253	282	177	218	216
	35 (40)	213	288	310	173	204	205
	Mean	**208.4**	**284.8**	**312.2**	**180.0**	**216.2**	**224.5**
	± SD	**± 14.3**	**± 21.6**	**± 21.3**	**± 13.7**	**± 23.7**	**± 24.3**
3000	21 (26)	197	259	300	170	200	210
	22 (27)	218	285	319	166	200	215
	23 (28)	229	300	336	172	209	213
	24 (29)	213	284	325	171	197	215
	25 (30)	217	302	360	198	249	261
	Mean	**214.8**	**286**	**328**	**175.4**	**211**	**222.8**
	± SD	**± 11.6**	**± 17.2**	**± 22.1**	**± 12.8**	**± 21.7**	**± 21.5**
5000	11 (16)	196	272	313	184	222	238
	12 (17)	212	291	329	189	226	234
	13 (18)	221	303	334	174	206	216
	14 (19)	219	286	323	164	200	210
	15 (20)	218	294	322	180	216	229
	Mean	**213.2**	**289.2**	**324.2**	**178.2**	**214**	**225.4**
	± SD	**± 10.2**	**± 11.4**	**± 7.9**	**± 9.7**	**± 10.9**	**± 11.9**
8000	1 (6)	212	297	345	195	231	247
	2 (7)	201	272	314	179	204	234
	3 (8)	218	284	326	174	207	233
	4 (9)	191	257	305	191	221	234
	5 (10)	**209**	279	317	181	223	233
	Mean	**206.2**	**277.8**	**321.4**	**184.0**	**217.2**	**236.2**
	± SD	**± 10.5**	**± 14.8**	**± 15.2**	**± 8.7**	**± 11.4**	**± 6.1**

§ = animal No. of males and (females)

Note: Standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY
No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female rats was greater than 8000 mg/kg bw.

Assessment and conclusion by applicant:
The study is performed according to OECD guideline 401, which is cancelled. Nevertheless, except for deviations provided above, the study is in concordance to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The acute oral LD_{50} is above 8000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of Glyphosate Technical in male and female rats is above 8000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015). It is noted, however, that the study is considered acceptable (reliable with restrictions) due to the noted deviations.

B.6.2.1.29. *Study 29*

Data point:	CA 5.2.1/029
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: Acute oral toxicity (limit) test in rats
Report No	5883
Document No	Not reported
Guidelines followed in study	OECD, EEC, EPA guidelines. (Guideline numbers are not specified in the report)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. Purity of the test substance not provided. From the batch number a purity of 98.6 % was concluded. Number of air changes during housing of the animals lacking. Only average data on the maximum and minimum temperature. A significant impact on the study outcome is not expected.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

Executive Summary
The acute oral toxicity of glyphosate technical was investigated in male and female rats (5 animals/sex/group) of the Sprague-Dawley strain. Test substance, suspended in 0.5 % Carboxymethylcellulose, was administered by oral gavage to each animal at a dosage of 5000 mg/kg bw and constant dose volume of 10 mL/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study. Clinical signs noticed were piloerection, reduced activity and ataxia. Changes of body weight were in the normal range. No mortality occurred. No abnormal necropsy findings were noted. The acute oral LD50 was determined to be: LD50, oral, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Glyphosate
Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-JaK-25-1
Purity:	Not specified in the study report; Batch 206-JaK-25-1 reported with 98.6 %, see also [redacted] 1991 (CA 5.2.1/025).
Stability of test compound:	Not specified
2. Vehicle and/or positive control:	0.5 % Carboxymethylcellulose (CMC)
3. Test animals:	
Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	6 – 8 weeks
Sex:	Male and female
Weight at dosing:	136 – 176 g
Acclimation period:	7 days
Diet/Food:	Expanded Rat and Mouse Maintenance Diet, *ad libitum* (except 18 hours prior to dosing and 3-4 hours after dosing)
Water:	Tap water, *ad libitum*
Housing:	Maximum of 5 animals/sex/cage in Polypropylene cages with mesh floors
Environmental conditions:	Temperature: 19 – 22 °C Humidity: 48 % (Average) Air changes: Not specified 12-hour light / dark cycle (light during 07:00 – 19:00)

B. STUDY DESIGN AND METHODS

In life dates: 25/05/1989 to 22/06/1989 (Arrival of the animals to termination of the study)
07/06/1989 to 22/06/1989 (experimental phase – without acclimation period)

Animal assignment and treatment:
Rats were housed by sex (5 males and 5 females) and starved for approximately 18 hours prior to dosing. Test material was suspended in 0.5 % CMC at concentration of 500 mg/mL and administered by a single oral gavage at dose level of 5000 mg/kg bw with an application volume of 10 mL/kg bw. Animals were starved for a further 3 – 4 hours. All animals were observed for clinical signs of toxicity at several time points on the day of administration and at least once a day, thereafter. Body weights were recorded prior to administration, on day 7 and prior to sacrifice on day 14. At study termination all animals were sacrificed followed by gross necropsy examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred during the 14-day observation period after administration.

B. CLINICAL OBSERVATIONS

The observed clinical signs were piloerection, reduced activity and ataxia. Females recovered after day 7, while clinical signs in males lasted until after day 9. All clinical signs are listed in the table below.

Table B.6.2.1.29-1: Glyphosate Technical: Acute oral toxicity (limit) test in rats [redacted] 1989): Clinical observations

Table B.6.2.1.29-1: Glyphosate Technical: Acute oral toxicity (limit) test in rats [redacted] 1989): Clinical observations

Dose group	**5000 mg/kg bw**												
Sex	**Males**							**Females**					
Time after treatment	**1 min**	**30 min**	**1 h**	**2 h**	**1-8 d**	**9 d**	**10–14 d**	**1 min**	**30 min**	**1 h**	**2 h**	**1-7 d**	**8 - 14 d**
Total animals n examined	5	5	5	5	5	5	5	5	5	5	5	5	5
Clinical sign n	0	5	5	5	5	5	0	0	5	5	5	5	0
Piloerection n	0	5	5	5	5	0	0	0	5	5	5	5	0
Reduced activity n	0	0	5	5	5	5	0	0	0	5	5	5	0
Ataxia n	0	0	0	0	0	5	0	0	0	0	0	0	0

C. BODY WEIGHT

Weight gain of all animals was within the normal range. Individual body weights are depicted in the table below.

Table B.6.2.1.29-2: Glyphosate Technical: Acute oral toxicity (limit) test in rats [redacted] 1989): Body weight and body weight gain

Dose level	**5000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0-14**	**0-14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
1 M (6 F)	176	239	284	136	168	182	108	46
2 M (7 F)	166	226	272	138	167	182	106	44
3 M (8 F)	169	255	316	151	192	202	147	51
4 M (9 F)	166	238	282	154	186	208	116	54
5 M (10 F)	162	232	269	137	165	179	107	42
Mean ± SD	**168 ± 5**	**238 ± 11**	**285 ± 19**	**143 ± 9**	**176 ± 12**	**191 ± 13**	**117 ± 17**	**47 ± 5**

§ = animal No. of males and (females)

D. NECROPSY

No abnormal necropsy findings were noticed.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in male and female rats was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study was performed according to guidelines similar to the current OECD guideline 420 (2001). Due to the deviations and the limited reporting, the study is only considered supplementary. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of Glyphosate Technical (PMG) in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.30. *Study 30*

Data point:	CA 5.2.1/030
Report author	[redacted]
Report year	1989

Report title	Acute oral toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent of 46 % of N-phosphonomethylglycine acid) in rats
Report No	PRO439 / 238050
Document No	Not reported
Guidelines followed in study	OECD 401 (1987), EEC Guidelines B.1 (1984)
GLP	Yes
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of Glyphosate Technical (IPA salt 62 % in water equivalent of 46 % of glyphosate) was investigated in male and female rats (KFM-Han. Wistar, 9-11 weeks at dosing, 191-212 (males) and 173-189 g (females) at dosing, 5 animals/sex/dose). The test article was dosed as such. The dose level was 2000 mg/kg bw. Administration occurred orally by means of a gavage at a volume of 2 mL/kg bw. Rats were weighed prior to dosing and at 8 and 15 days after dosing. Clinical signs were recorded for 14 days following dosing before rats were sacrificed. Afterwards, all rats were subjected to necroscopy.
Short description of results:	No deaths occurred. Clinical findings were not noted. Body weight gain was normal. No abnormalities were detected at necroscopy. The LD_{50} for Glyphosate Technical (IPA salt 62 % in water equivalent of 46 % of glyphosate) was determined to be greater than 2000 mg/kg bw in male and female mice.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, Category 4a **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered to be valid. The LD_{50} for Glyphosate Technical was determined to be greater than 2000 mg/kg bw in male and female rats. This in in line with the conclusion from the first evaluation (DAR, 1998).
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** see above.

B.6.2.1.31. *Study 31*

Data point:	CA 5.2.1/031
Report author	[redacted]
Report year	1988
Report title	Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats
Report No	88.2053.007
Document No	Not reported
Guidelines followed in study	US EPA 81-1, equivalent to OECD 420 (2001)
Deviations from current test guideline (OECD 420, 2001)	Yes, both sexes used instead of one sex (usually females), observation three times at the first day at unknown points in time and twice a day thereafter instead of once. Animals not fasted after dosing. The clinical signs per

	individual animal are not reported in the report. A significant impact on the study outcome is not expected.
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 to be acceptable.

Executive Summary
The test substance, glyphosate, was evaluated for its acute oral toxicity potential in male and female Sprague-Dawley rats when administered as a gavage dose at level of 5000 mg/kg bw. No mortalities occurred. Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen. Body weight gain was noted for all animals. No internal abnormalities were noted during gross necropsy examination of the animals. The acute oral LD_{50} was determined to be: LD_{50}, oral, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76%
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	♂ 300 – 332 g; ♀ 217 – 222 g
Acclimation period:	At least 5 days
Diet/Food:	NIH Open Formula 07 Rat and Mouse Diet, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum* (except when fasted overnight prior to dosing)
Water:	Tap water, *ad libitum*
Housing:	Wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 ºC Humidity: 40 – 70% Air changes: Not specified Light cycle: 12 hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1988-04-05 to 1988-04-19

Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose level of 5000 mg/kg bw by oral

gavage as a 50 % w/v aqueous suspension. Observations for mortality and signs of toxicity were made three times on the day of dose administration and twice daily thereafter. Body weights were recorded prior dose administration on study day 1, and on days 8 and 15 (terminal sacrifice). A gross necropsy was performed on all animals at the time terminal sacrifice (day 15) and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred.

B. CLINICAL OBSERVATIONS

Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen (see table below).

Table B.6.2.1.31-1: Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats ([redacted], 1988): Clinical observations during the study.

Dose group		**5000 mg/kg bw**										
Sex		**Males**								**Females**		
Days after treatment		1	2	3	4	5	6	7	8-15	1	2	3	4-15
Total animals examined	**n**	5	5	5	5	5	5	5	5	5	5	5	5
Clinical sign	**n**	0	5	1-2[#]	1	1	1	0	1	0	5	0	1
Hair loss	n	0	0	0	1	1	1	0	1	0	0	0	1
Diarrhea	n	0	5	1	0	0	0	0	0	0	5	0	0
Wet abdomen	n	0	3	1	0	0	0	0	0	0	2	0	0

= From the reported data it cannot be concluded if 1 or 2 animals were affected in total

C. BODY WEIGHT

Body weight gain was noted for all animals (see table below).

Table B.6.2.1.31-2: Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats ([redacted], 1988): Body weight development

Dose group	**5000 mg/kg bw**					
Sex	**Males**			**Females**		
Time after treatment	**Day 1[a]**	**Day 8**	**Day 15**	**Day 1[a]**	**Day 8**	**Day 15**
Animal No.[§]	**Body weight (g)**					
70011 (70016)	310	357	394	220	232	243
70012 (70017)	332	371	411	222	245	255
70013 (70018)	300	349	382	221	241	250
70014 (70019)	309	342	380	217	248	258
70015 (70020)	318	363	389	217	243	253
Mean (± SD)	**313.8 ± 12.0**	**356.4 ± 11.4**	**391.2 ± 12.4**	**219.4 ± 2.3**	**241.8 ± 6.1**	**251.8 ± 5.7**

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.
§ = animal No. of males and (females), a= fasted body weight

D. NECROPSY

No internal abnormalities were noted during gross necropsy examination of the animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate in male and female rats was greater 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study is in accordance to the current OECD guideline 420 (2001). The outcome can be therefore reported as valid. The acute oral LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute oral LD_{50} of glyphosate in male and female rats is above 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.32. *Study 32*

Data point:	CA 5.2.1/032
Report author	[redacted]
Report year	1987
Report title	Acute oral LD_{50} study of MON-8750 in Sprague-Dawley rats
Report No	[redacted] 86-431/9308A
Document No	Not reported
Guidelines followed in study	EPA OPP 81-1 (Acute Oral Toxicity)
Deviations from current test guideline (OECD 420, 2001)	Both sexes, each with 5 animals per dose group instead of one sex (usually females) with 5 animals per dose group. Number of air changes during housing of the animals lacking. Stability of test compound not reported. No fasting after treatment. Age of the rats not provided. Individual clinical and necropsy findings were not reported. These deviations are not expected to significantly impact the study outcome.
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 to be acceptable.

Executive Summary

The acute oral toxicity of glyphosate (MON 8750) was investigated in 1 female and 1 male rat in a limit test using a dose level of 5000 mg/kg bw. Subsequently, a dose-range study was conducted to determine suitable dose levels for the main study. On basis of the dose-range study and the limit test, 5 male and 5 female rats were orally dosed with the test substance at dose levels of 2222, 5000 and 7500 mg/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study. Mortality did not occur in the low dose group. However, 6/10 animals died in the middle dose group and 9/10 animals died in the high dose group. Clinical signs included but were not limited to ataxia, laboured breathing and reduced activity. The findings were especially prominent in the middle and high dose group. Changes of body weight were in the normal range. Findings at necropsy concerned but were not limited to the lungs, the cecum and the intestines in the middle and high dose group. No findings were reported for the low dose group. On basis of the limit test and the main study, the acute oral LD50 was calculated to be:

LD_{50}, oral, female and male rat = 4613 mg/kg bw (95 % confidence interval (CI) 3511–5716 mg/kg bw)
LD_{50}, oral, female rat = 2222 < 5000 mg/kg bw
LD_{50}, oral, male rat = 5904 mg/kg bw (95 % CI 3420 – 8388 mg/kg bw)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: MON8750
Description: White powder
Lot/Batch #: XLG-255

Purity:	90.8%
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Distilled/deionised water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	222 – 311 g (limit test), 217 – 297 g (main study)
Acclimation period:	At least 5 days
Diet/Food:	Open formula, certified diet, Zeigler Brothers, *ad libitum* (except for fasting period overnight before dosing)
Water:	Tab water, *ad libitum*
Housing:	Wire mesh cages
Environmental conditions:	Temperature: 20 – 24 °C Humidity: 40 – 70 % Air changes: Not specified

B. STUDY DESIGN AND METHODS

In life dates: Not reported

Animal assignment and treatment:
A limit test was conducted by oral gavage administration. The test substance was administered after overnight fasting to five female and five male rats at a dose level of 5000 mg/kg bw. 6/10 animals died.
Furthermore, a dose-range finding study was performed after overnight fasting on one female and one male rat per dose group (500, 1000, 3000, 5000 and 7000 mg/kg bw) to determine appropriate dose levels for the main study. One animal died in the highest dose group. The limit test should be used for determination of an LD_{50} together with the main study. Based on this and the dose-range finding study, dose levels of 2222 and 7500 mg/kg bw were chosen for the main study.
During the main study, 5 male and 5 female rats per dose group were fasted overnight and dosed by oral gavage with the test substance. Clinical observations and mortality were recorded three times on the day of dosing and twice daily thereafter for the duration of the study. Body weights were measured on day 1 prior to dosing, day 8 and day 15 or at death. All animals were sacrificed at the end of the study and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality occurred in one male rat and all 5 female rats during the limit test (5000 mg/kg bw). One animal of the highest dose group (7000 mg/kg bw) died during the dose-range finding study (results not shown). During the main study, mortality occurred with 4 males and all 5 females in the highest dose group (7500 mg/kg bw).

Table B.6.2.1.32-1: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats [redacted] 1987): Mortality during limit test and main study

Dose level (mg/kg bw)	Cumulative mortality at Day[1]				
Males	**1**	**2**	**3**	**4**	**5-15**
2222	0/5	0/5	0/5	0/5	0/5
5000[2]	0/5	0/5	1/5	1/5	1/5
7500	1/5	3/5	4/5	4/5	4/5
Females					
2222	0/5	0/5	0/5	0/5	0/5
5000[2]	1/5	4/5	4/5	5/5	5/5

Table B.6.2.1.32-1: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats [redacted] 1987): Mortality during limit test and main study

Dose level (mg/kg bw)	Cumulative mortality at Day[1]				
7500	0/5	4/5	5/5	5/5	5/5

1 = Number dead animals / number of exposed animals per group, 2 = Dose level administered in limit test

Based on the mortality, the acute oral LD_{50} in males was calculated to be 5904 mg/kg bw with a 95 % CI of 3420 – 8388 mg/kg bw. The acute oral LD_{50} for females was calculated to be between 2222 and 5000 mg/kg bw. The combined LD_{50} was calculated to be 4613 mg/kg bw with a 95 % CI of 3511 – 5716 mg/kg bw.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were observed during the limit test (5000 mg/kg bw) and in the high dose group of the main study (7500 mg/kg bw) and included but were not limited to ataxia, decreased activity, diarrhea and labored breathing (see table below).

Table B.6.2.1.32-2: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats [redacted] 1987): Clinical observations recorded during the study in males and females

Dose level (mg/kg)	2222	5000[1]	7500	2222	5000[1]	7500
Sex	Males			Females		
Clinical observation	Incidence[2] (study day)					
Ataxia		2/5 (1)	2/5 (1) 1/2 (2) 1/1 (3-5)		1/5 (1) 1/1 (3)	3/5 (1) 1/1 (2)
Decreased activity		5/5 (1)	3/5 (1) 2/2 (2) 1/1 (3-6)		4/5 (1) 1/2 (2) 1/1 (3)	5/5 (1) 1/1 (2)
Diarrhea	2/5 (1)	3/5 (1) 2/5 (2) 1/4 (3,4)	2/5 (1) 2/2 (2) 1/1 (3,4)	4/5 (1)	2/5 (1) 2/2 (2) 1/1 (3)	1/1 (2)
Hair loss – base of tail		1/4 (4-11)				
Hair loss – abdomen		1/4 (5-15)				
Gasping						1/1 (2)
Hypothermia						1/1 (2)
Laboured breathing			2/2 (2) 1/1 (3-5)		1/5 (1) 1/1 (3)	4/5 (1) 1/1 (2)
Lacrimation						1/1 (2)
Pale						1/1 (2)
Rales					1/1 (3)	
Sores- base of tail		1/4 (5-7)				
Wet abdomen		1/4 (3,4)				1/1 (2)
Dark material around nose		1/4 (3)				
Dark material around left eye		1/4 (3)				

1 = Dose level administered in limit test, 2 = number of animals with observed clinical signs / number of animals examined with study day(s) in parenthesis at which the clinical signs were observed.

C. BODY WEIGHT

Treatment with the test substance did not have an effect on body weights in surviving animals (see table below).

Table B.6.2.1.32-3: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats [redacted] 1987): Summary of body weights recorded during limit test and main study in males and females

Day		1[2]	8	15	1	8	15
Sex		Males			Females		
Dose level (mg/kg bw)	Animal No.[§]	Body weight [g]					

Table B.6.2.1.32-3: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats [redacted] 1987): Summary of body weights recorded during limit test and main study in males and females

Day		**1[2]**	**8**	**15**	**1**	**8**	**15**
Sex		**Males**			**Females**		
Dose level (mg/kg bw)	**Animal No.[§]**	**Body weight [g]**					
2222	93080051 (93080056)	297	361	385	234	264	267
	93080052 (93080057)	255	298	316	236	276	283
	93080053 (93080058)	251	297	328	217	246	258
	93080054 (93080059)	249	340	378	219	241	250
	93080055 (93080060)	258	301	316	217	239	245
Mean		**262.0**	**319.4**	**344.6**	**224.6**	**253.2**	**260.6**
± SD		**± 19.9**	**± 29.4**	**± 34.1**	**± 9.5**	**± 16.1**	**± 15.0**
5000[1]	93080021 (93080026)	310	372	397	226	-	-
	93080022 (93080027)	303	-	-	237	-	-
	93080023 (93080028)	311	326	363	222	-	-
	93080024 (93080029)	310	348	376	228	-	-
	93080025 (93080030)	299	334	360	230	-	-
Mean		**306.6**	**345.0**	**374.0**	**228.6**	**-[4]**	**-[4]**
± SD		**± 5.3**	**± 20.2**	**± 16.8**	**± 5.5**	**-[4]**	**-[4]**
7500	93080061 (93080066)	291	-	-	223	-	-
	93080062 (93080067)	291	-	-	219	-	-
	93080063 (93080068)	281	311	355	222	-	-
	93080064 (93080069)	269	-	-	217	-	-
	93080065 (93080070)	249	-	-	218	-	-
Mean		**276.2**	**-[3]**	**-[3]**	**219.8**	**-[4]**	**-[4]**
± SD		**± 17.7**	**-[3]**	**-[3]**	**± 2.6**	**-[4]**	**-[4]**

1 = Dose level administered in limit test, 2 = Fasted body weights,
3 = Group mean ± Standard deviation not calculated, insufficient number of animals.
4 = Group mean ± Standard deviation not calculated, 100 % mortality.
§ = animal No. of males and (females)
- = animal died
Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

During necropsy, no abnormal changes on organs were noticed in the low dose group. Findings during necropsy in the middle and high dose group concerned the lungs, stomach and intestines. Observed findings in the stomach, most pronounced at mid and high dose levels are most likely due to the irritating properties of glyphosate. In addition, in short-term studies with rats and mice irritation with diarrhoea was observed in the gastro-intestinal tract. All findings are listed in the table below.

Table B.6.2.1.32-4: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats [redacted] 1987): Findings during gross necropsy

Dose level (mg/kg bw)	**2222**	**5000[1]**	**7500**	**2222**	**5000[1]**	**7500**
Sex	**Males**			**Females**		
Tissue/ Finding	**Incidence[2]**					
Cecum - contains black substance - contains red fluid			1/5			1/5
Intestines – contain red fluid			1/5			1/5
Lungs- dark/red areas		2/5			2/5	
Stomach - contains dark fluid - red areas glandular mucosa		1/5	4/5 2/5		1/5 1/5	5/5

Table B.6.2.1.32-4: Acute oral LD50 study of MON-8750 in Sprague-Dawley rats ([redacted], 1987): Findings during gross necropsy

- contains blood-like substance					1/5	

1 = Dose level administered in limit test, 2 = number of animals with observed findings / number of animals examined.

III. CONCLUSIONS

The combined oral LD_{50} (males and females) of glyphosate (MON8750) in rats was calculated to be 4613 mg/kg bw with a 95 % CI of 3511 – 5716 mg/kg bw.
The acute oral LD_{50} in males was calculated to be 5904 mg/kg bw with a 95 % CI of 3420 – 8388 mg/kg bw. The acute oral LD_{50} in females was calculated to be between 2222 and 5000 mg/kg bw.

Assessment and conclusion by applicant:
The study was performed according to a national guideline similar to the current OECD guideline 420 (2001). Therefore, the outcome can be reported as valid. The combined acute oral LD_{50} is 4613 mg/kg bw (95 % CI 3511 – 5716 mg/kg bw). The acute oral LD_{50} in males was calculated to be 5904 mg/kg bw (95 % CI 3420 – 8388 mg/kg bw). The acute oral LD_{50} in females was calculated to be between 2222 and 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The combined acute oral LD_{50} of glyphosate (MON8750) is 4613 mg/kg bw (95 % CI 3511 – 5716 mg/kg bw). The acute oral LD_{50} of glyphosate (MON8750) in males was calculated to be 5904 mg/kg bw (95 % CI 3420 – 8388 mg/kg bw). The acute oral LD_{50} of glyphosate (MON8750) in females was calculated to be between 2222 and 5000 mg/kg bw. This is in line with the conclusion from the previous evaluation (RAR, 2015).

B.6.2.1.33. *Study 33*

Data point:	CA 5.2.1/033
Report author	[redacted]
Report year	1987
Report title	Acute oral toxicity study of MON 8722 in Sprague-Dawley rats
Report No	[redacted]-86-430/9307A
Document No	Not reported
Guidelines followed in study	EPA Guidelines, Subdivision F
GLP	Yes
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of glyphosate (MON 8722) was investigated in male and female rats (Sprague-Dawley, age unknown, 224-316 g, 5 animals/sex/dose). The test article was diluted in water. The dose level was 5000 mg/kg bw. Administration occurred orally by gavage. The dose volume is unknown. Rats were weighed prior to dosing and at 8 and 15 days after dosing. Clinical signs were recorded three times on the day of dosing and twice daily for 14 days following dosing before rats were sacrificed. Afterwards, all rats were subjected to necroscopy.
Short description of results:	No deaths occurred. Clinical findings were ataxia, decreased activity, diarrhoea and rectal sores. Animals gained weight over 14 days. No abnormalities were detected at necroscopy. The LD_{50} for glyphosate (MON 8722) was determined to be greater than 5000 mg/kg bw in male and female rats.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000; Category 4a

	Conclusion AGG: In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered to be valid. The LD_{50} for glyphosate (MON 8722) was determined to be greater than 5000 mg/kg bw in male and female rats. This in in line with the conclusion from the first evaluation (DAR, 1998).
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** see above.

B.6.2.1.34. *Study 34*

Data point:	CA 5.2.1/034
Report author	[redacted]
Report year	1987
Report title	Acute oral toxicity of 64 % SN750721 technical liquid in mice
Report No	[redacted] 8ao1
Document No	Not reported
Guidelines followed in study	Not reported.
GLP	Not reported. Assumed to be non-GLP.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of 64% SN750721 technical liquid was investigated in male and female mice (ICR strain, 4 weeks, 25.0 ± 1.5 g, 5 animals/sex/dose). Test doses were 4125, 4625, 5125, 5625, and 6125 mg/kg bw (dose volume unknown). The test item was administered undiluted. Each test was repeated three times. Clinical signs were recorded 0.5, 1, 2, 4 hours after dosing and afterwards once daily for 14 days before rats were sacrificed. Afterwards, all rats were subjected to necroscopy. Body weights were not recorded.
Short description of results:	LD_{50} = 4373 mg/kg bw (confidence interval: 4144-4644 mg/kg bw) Dose related mortality in all IPA-treated groups. Mice died within three days after administration. Clinical signs in poisoned mice were immobility, tremor, hyperaemia of the ears. No gross abnormalities were found at necroscopy.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000; Category 4a **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. Substantial information is missing in the study report such as body weight data and dose volume, therefore the study is not considered reliable for evaluation. An LD_{50} is not derived. This stands in contrast to the first evaluation (DAR, 1998) where the study was accepted.
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** see above.

B.6.2.1.35. *Study 35*

Data point:	CA 5.2.1/035
Report author	[redacted]
Report year	1987
Report title	Acute oral toxicity of 41 % SN750721 solution in mice
Report No	[redacted]58ao2
Document No	Not reported
Guidelines followed in study	Not reported.
GLP	Not reported. Assumed to be non-GLP.
Previous evaluation	Study was not part of the previous evaluation (RAR, 2015) but it was considered in the first evaluation (DAR, 1998).
Short description of study design and observations:	The acute oral toxicity potential of 41% SN750721 technical liquid was investigated in male and female mice (ICR strain, 4 weeks, 25.3 ± 1.8 g, 5 animals/sex/dose). Test doses were 3125, 3625, 4125, 4625, and 5125 mg/kg bw (dose volume unknown). The test item was administered undiluted. Each test was repeated three times. Clinical signs were recorded 0.5, 1, 2, 4 hours after dosing and afterwards once daily for 14 days before rats were sacrificed. Afterwards, all rats were subjected to necroscopy. Body weights were not recorded.
Short description of results:	LD_{50} = 3669 mg/kg bw (confidence interval: 3489 – 3858 mg/kg bw) Dose related mortality in all IPA-treated groups. Mice died within three days after administration. Clinical signs in poisoned mice were immobility, tremor, hyperemia of the ears. No gross abnormalities were found at necroscopy.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000; Category 4a **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. Substantial information is missing in the study report such as body weight data and dose volume, therefore the study is not considered reliable for evaluation. An LD_{50} is not derived. This stands in contrast to the first evaluation (DAR, 1998) where the study was accepted.
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** see above.

B.6.2.1.36. *Study 36*

Data point:	CA 5.2.1/036
Report author	[redacted]
Report year	1983
Report title	The acute oral toxicity (LD_{50}) to albino mice with glyphosate (tech) of [redacted]
Report No	TOX95-51812
Document No	Not reported
Guidelines followed in study	None

Deviations from current test guideline (OECD 420 (2001))	Five animals of both sexes, high doses used, age not specified, housing conditions not specified, no gender specific differentiation of mortality data, body weights not recorded. Fasting period prior to dosing was overnight instead of 3-4 hours for mice.
Previous evaluation	Study not included in the RAR (2015). Study considered supplementary only in the DAR (1998).
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered to be not acceptable due to the deviations noted above. In addition, the study was not conducted according to GLP although it is recognised that GLP was not compulsory at the time the study was performed.

Executive Summary

The test substance, glyphosate technical, was evaluated for its acute oral toxicity potential. 5 female and 5 male mice were used per dose group. The test substance was administered as a single oral gave at levels of 0, 2000, 3000, 4000, and 5000 mg/kg bw. Clinical signs included ataxia and loss of muscle tone at 3000, 4000 and 5000 mg/kg bw, which were no longer observed after 24-hours. Mortality occurred in the same dose groups. 20 %, 50 % and 70 % of the animals died at 3000, 4000 and 5000 mg/kg bw, respectively. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be: LD_{50}, oral, female and male mice = 4000 mg/kg bw (CI: 3330 mg/kg bw – 4800 mg/kg bw).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical
Description:	White amorphous powder
Lot/Batch #:	R&D sample (9-7-83)
Purity:	95 %
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: No information provided

3. Test animals:

Species:	Mice
Strain:	Kasauli
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	20 – 25 g
Acclimation period:	Not specified
Diet/Food:	Pelleted feed (supplied by Hindustan Lever Ltd., Bombay), *ad libitum*, except for a fasting period the night before dosing
Water:	Not specified, *ad libitum*
Housing:	Groups of 5 / sex
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified 12-hour light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No information provided
Finalisation date: 12/10/1983

Animal assignment and treatment:
Glyphosate technical was tested in a dose-range finding study with 5 mice/sex/group. Based on the mortality in the preliminary study, following dose groups were chosen for the main study: 0, 2000, 3000, 4000, and 5000 mg/kg bw. Prior to dosing, all animals were fasted overnight. The test substance was administered to 5 mice/sex/group by a single oral gavage. The animals were observed over the study period of 15 days for signs of toxicity and mortality.

II. RESULTS AND DISCUSSION

A. MORTALITY

2/10 animals died at 3000 mg/kg bw 24-hours after treatment. A total of 5/10 animals and 7/10 animals died between 8 hours and 2 days after treatment at 4000 and 5000 mg/kg bw, respectively. For details see table below.

Table B.6.2.1.36-1: The acute oral toxicity (LD_{50}) to albino mice with glyphosate (tech) of [redacted] ([redacted] 1983): Mortality for all dose groups

Dose level (mg/kg bw)	Mortality*	Mortality rate (%)
0	0 / 10	0
2000	0 / 10	0
3000	2 /10 (24 h)	20
4000	1 / 10 (8 h); 2 / 10 (24 h); 2 / 10 (2 d)	50
5000	2 / 10 (8 h); 3 / 10 (24 h); 2 / 10 (2 d)	70

* = No. of deaths/No. of animals examined (Hours or Day of death)

B. CLINICAL OBSERVATIONS

No clinical signs were observed in the control group and at 2000 mg/kg bw. Sign observed in all mice at 3000, 4000 and 5000 mg/kg bw included ataxia and loss of muscle tone between 4 and 24-hours after treatment. No clinical signs were observed thereafter (see table below).

Table B.6.2.1.36-2: The acute oral toxicity (LD_{50}) to albino mice with glyphosate (tech) of [redacted] ([redacted], 1983): Individual clinical observations

Clinical observation	**Incidence[1]**				
Dose level (mg/kg bw)	**0**	**2000**	**3000**	**4000**	**5000**
Sex	**Males**				
Ataxia	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)
Loss of muscle tone	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)
Sex	**Females**				
Ataxia	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)
Loss of muscle tone	0	0	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)	5/5 (4, 8, 24 h)

1 = number of animals with observed clinical signs / number of animals examined within the dose group and hour(s) in parenthesis at which the clinical signs were observed.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities in deceased

and surviving animals.

III. CONCLUSIONS

The oral LD_{50} of glyphosate technical in mice was estimated to be 4000 mg/kg bw (CI: 3330 mg/kg bw – 4800 mg/kg bw).

Assessment and conclusion by applicant:
Due to the deviations of the study and the fact that it was not performed according to any guideline and not according to GLP, the study provides supplementary information on acute oral toxicity of glyphosate, only.

Assessment and conclusion by RMS:
The study is not considered acceptable for evaluation due to the deviations noted above. No LD_{50} is derived.

B.6.2.1.37. *Study 37*

Data point:	CA 5.2.1/037
Report author	[redacted]
Report year	1983
Report title	The acute oral toxicity (LD50) to rats with glyphosate (tech.).
Report No	Not given
Document No	Not reported
Guidelines followed in study	None
GLP	No (GLP was not compulsory at the time the study was performed)
Previous evaluation	Study not included in the RAR (2015). Study considered supplementary only in the DAR (1998).
Short description of study design and observations:	Not available
Short description of results:	Slight ataxia (at 5000 mg/kg bw)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, Category 4a. **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report, however, considering that: (i) the study was not conducted according to any guideline; (ii) the study was not conducted according to GLP, although not compulsory at that time; (iii) the study was not considered in the RAR (2015) and only considered supportive in the DAR (1998); and (iv) no substantial new information is obtained from this study, the AGG did not summarise the study in more detail. It is noted that the quality of the study report is very low and at times unreadable. Moreover, very little details are provided on the conduct of the study. It is not considered adequate to derive an LD_{50} from this study. However, it is noted that no mortality was observed in the study up to the highest dose tested (5000 mg/kg bw).
Reasons why the study report is not available for submission	**Conclusion GRG:** The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL **Conclusion AGG:** see above.

B.6.2.1.38. *Study 38*

Data point:	CA 5.2.1/038

Report author	[redacted]
Report year	1981
Report title	Acute oral toxicity of MON 0139 to rats
Report No	800257
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 420, 2001)	Both sexes used instead of one sex (usually females). Purity and stability of test compound not reported. Number of air changes during housing of the animals lacking. Environmental conditions not reported. Type of diet not reported. Age of the rats and acclimation period not reported. Animals were not fasted after dosering. Individual body weights and necropsy findings not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered to be not acceptable due to the multiple deviations noted above.

Executive Summary
The acute oral toxicity of glyphosate (MON 0139) was investigated in 5 male and 5 female rats of the Sprague-Dawley strain. The test substance was administered undiluted by oral gavage to each animal at a dosage of 5000 mg/kg bw. Mortality, body weight and clinical signs were recorded during the subsequent 14 days. All animals were subjected to a gross necropsy at the end of the study. One animal died on the day of dosing. This was not attributed to the substance. No clinical signs were noticed. Changes of body weight were in the normal range. Pale kidneys (2 animals) and bilateral hydronephrosis (1 animal) were observed during necropsy. The acute oral LD50 was determined to be: LD50, oral, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: MON 0139
Description: Amber liquid
Lot/Batch #: SSRT-11012
Purity: Not specified
Stability of test compound: Not specified

2. Vehicle and/or positive control: Undiluted

3. Test animals:

Species: Rat
Strain: Sprague-Dawley (Crl:CD® (SD)BR)
Source: [redacted]
Age: Not specified
Sex: Male and female
Weight at dosing: 256 – 276 g (males), 178 – 196 g (females)
Acclimation period: Not specified
Diet/Food: Not specified, *ad libitum* (except for fasting period overnight before dosing)

Water:	Not specified, *ad libitum*	
Housing:	Individually housed	
Environmental conditions:	Temperature:	Not specified
	Humidity:	Not specified
	Air changes:	Not specified
	light/dark cycle:	Not specified

B. STUDY DESIGN AND METHODS

In life dates: 18/09/1980 to 02/10/1980 (Experimental phase)

Animal assignment and treatment:
A group of 5 male and 5 female rats was fasted overnight and dosed with the test substance at a concentration of 5000 mg/kg bw. After treatment, animals were housed individually and observed for mortality and clinical signs three times within the first 8 hours after dosing and twice daily (morning and afternoon) thereafter. Surviving animals were sacrificed after 15 days and were subjected to necropsy. Body weights were recorded immediately before dosing and on day 7 and 14 during the study. One animal died due to flawed administration.

II. RESULTS AND DISCUSSION

A. MORTALITY
One male animal died on the day of dosing probably as a result of an oesophageal rupture as test material was found in the thoracic cavity.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14 days observation period.

C. BODY WEIGHT
All animals gained weight during the study (see table below).

Table B.6.2.1.38-1: Acute oral toxicity of MON 0139 [redacted] 1981): Mean body weight

Dose level	**5000 mg/kg bw**					
Sex	**Males**			**Females**		
Day	**0**	**7**	**15**	**0**	**7**	**15**
Body weight [g]*						
Mean	267	339	378	188	235	250

* No body weight data of single animals reported.

D. NECROPSY
During necropsy, pale coloured kidneys were noticed in two females and bilateral hydronephrosis was noticed in one female.

III. CONCLUSIONS

The oral LD_{50} of glyphosate (MON 0139) in male and female rats was greater than 5000 mg/kg bw.

Assessment and conclusion by applicant:
Due to the deviations of the study and the fact that it was not performed according to GLP, the study provides supplementary information on acute oral toxicity of glyphosate, only.

Assessment and conclusion by RMS:
The study is not considered acceptable for evaluation due to the deviations noted above. No LD_{50} is derived. This stands in contrast to the RAR where it seems that the study was accepted.

B.6.2.1.39. *Study 39*

Data point:	CA 5.2.1/039
Report author	[redacted]
Report year	1979
Report title	Acute Oral Toxicity Study In Rats
Report No	[redacted]-77-428
Document No	Not reported
Guidelines followed in study	None (pre-guideline), similar to OECD 420 (2001)
Deviations from current test guideline (OECD 420 (2001)	5 animals per sex used, high doses, animals not fasted after dosing, for males and females clinical signs were provided not separately, age of the animals not specified, environmental conditions not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (pre-GLP)
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered to be not acceptable due to the multiple deviations noted above.

Executive Summary
The test substance, glyphosate, was evaluated for its acute oral toxicity potential in male and female Wistar strain albino rats when administered as a gavage dose at levels of 2500, 3500, 5000, 7000, and 9900 mg/kg bw (n=5 per sex per dose level). Mortalities at dose levels of 2500, 3500, 5000, 7000, and 9900 mg/kg bw were 1/10, 1/10, 3/10, 8/10, and 10/10, respectively. Clinical signs included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and faecal staining of the abdomen. For the 2500, 3500, 5000 and 7000 mg/kg bw dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study. The gross necropsy conducted at termination demonstrated discoloured lungs, liver and/or kidneys for the 2500 mg/kg bw group, discoloured lungs, liver and/or kidneys or air filled intestines for the 3500 mg/kg bw group, no findings for the 5000 mg/kg bw group, and discoloured lungs, liver and/or kidneys, and air filled intestines for the 7000 mg/kg bw group. No 9900 mg/kg bw animals survived to necropsy. The acute oral LD_{50} was calculated to be: LD_{50}, oral, rat 5600 mg/kg bw, with 95 % confidence limits of 4900 to 6300 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Technical
Description: Fine white powder
Lot/Batch #: XHI-180
Purity: 99%
Stability of test compound: No data given in the report

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species: Rat
Strain: Wistar
Source: [redacted]
Age: Not specified
Sex: Male and female

Weight at dosing:	Males: 240 – 294 g, females: 225-288 g	
Acclimation period:	Not specified	
Diet/Food:	*ad libitum* (except when fasted for approximately 18 hours prior to dosing)	
Water:	*ad libitum*	
Housing:	Individually	
Environmental conditions:	Temperature:	Not specified
	Humidity:	Not specified
	Air changes:	Not specified
	Light cycle:	Not specified

B. STUDY DESIGN AND METHODS

In life dates: Not specified

Animal assignment and treatment:
Groups of five male and five female rats received the test material at a dose levels of 2500, 3500, 5000, 7000, and 9900 mg/kg body weight by oral gavage. The test material was administered by oral intubation as a 25 % w/v solution in distilled water. Observations for mortality and overt signs of effect were made at 0-2 and 4-6 hours following dosing and twice daily thereafter (early morning and late afternoon) for fourteen days. Body weights were recorded prior to fasting, on Day 7, and on Day 14 of the study. A gross necropsy was performed on all animals at the time of death or terminal sacrifice (Day 14). All abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortalities in the study are as indicated in the table below.

Table B.6.2.1.39-1: Acute Oral Toxicity Study In Rats [redacted] 1979): Mortality observed after treatment

Dose Level (mg/kg body weight)	Mortality/Total Number of Animals Dosed
2500	1/10
3500	1/10
5000	3/10
7000	8/10
9900	10/10

Note: males and females were not reported separately.

B. CLINICAL OBSERVATIONS

Clinical signs included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and faecal staining of the abdomen (see table below).

Table B.6.2.1.39-2: Acute Oral Toxicity Study In Rats [redacted] 1979): Clinical observations recorded after treatment during the study

Dose level [mg/kg bw]	**2500**	**3500**	**5000**	**7000**	**9900**
Clinical observation	**Incidence***				
Ataxia	1/10 (d1-3,4)	2/10 (h0-2) 1/9 (d7)	1/9 (d1) 1/8 (d7) 0/7 (d14)	1/3 (h0-6) 1/2 (d2) 0/2 (d3-4) 0/2 (d7,14)	1/2 (h0-2) 1/1 (h4-6)
Convulsion	1/10 (d3-4)	-	-	-	-
Muscle tremor	1/10 (d2)	1/10 (d2)	1/9 (d1)	-	1/2 (h0-2)
Red nasal discharge	1/10 (d2)	-	4-5 (h0-6) 1/9 (d1) 1/8 (d7)	-	-

Table B.6.2.1.39-2: Acute Oral Toxicity Study In Rats [redacted] 1979): Clinical observations recorded after treatment during the study

Dose level [mg/kg bw]	**2500**	**3500**	**5000**	**7000**	**9900**
Clinical observation	**Incidence***				
			0/7 (d14)		
Rales	-	-	1/10 (h4-6)	-	-
Clear oral discharge	-	-	3/10 (h0-2)	1/3 (d4-6)	1/2 (h0-2)
Lacrimation	-	-	-	1-2/3 (h0-6)	-
Urinary staining	1/10 (d3-4)	1/10 (d2) 3/10 (d3)	1/10 (h4-6) 5/10 (d1)	1-2/2 (d2)	-
General unhealthy appearance	1/10 (d3)	-	1/8 (d7)	-	1/2 (h0-2) 1/1 (h4-6)
Soft stool	-	1/10 (h0-4, d2)	2-3/10 (h0-6) 3-6/10 (d1) 1/8 (d7) 0/7 (d14)	1-2/2 (d2)	-
Piloerection	5/10 (h0-2) 7/10 (h4-6) 4-8/10 (d1-3) 1/9 (d7) 0/9 (d14)	5/10 (h0-6) 4-8/10 (d1-3) 2/9 (d7) 0/9 (d14)	5-6/10 (h0-6) 6/9 (d1) 0/8 (d2-4,7) 0/7 (d14)	3/3 (h0-6) 2/2 (d1-3) 0/2 (d4,7) 0/2 (d14)	1/1 (h4-6)
Prostration	1/10 (d3) 0/9 (d4,7,14)	-	-	-	1/2 (h0-2)
Lethargy	1/10 (h0-2) 2/10 (h4-6) 5-7/10 (d1) 2-4/10 (d2-3) 1-2/10 (d4) 0/9 (d7,14)	6/10 (h0-2) 4/10 (h4-6) 2-4/10 (d1-2) 1-2/10 (d3) 1/9 (d7) 0/9 (d14)	2/10 (h0-2) 4/10 (h4-6) 1-3/9-10 (d1) 0/8 (d2-4) 1/8 (d7) 0/7 (d14)	2/3 (h0-6) 2/2 (d1-2) 1/2 (d3) 0/2 (d4,7) 0/2 (d14)	1/2 (h0-2) 1/1 (h4-6)
Hyperactivity	-	1/10 (h0-2)	4/10 (d1)	-	-
Fecal staining	1/10 (d1); 1/10 (d3-4) 0/9 (d7,14)	1/10 (h4-6); 1/10 (d2-3) 1/9 (d7) 0/9 (d14)	2-3/10 (h0-6) 7/9 (d1) 0/8 (d2-3) 1/8 (d4,7) 0/7 (d14)	1-2/2 (d2-3) 0/2 (d4,7) 0/2 (d14)	-
Hyperpnea	-	-	1/8 (d7)	-	-
Squinting eyes	-	-	-	2-3/3 (h0-6)	-
Dyspnea	-	-	-	1/2 (d1)	-
No observed abnormality	3-5/10 (h0-6) 1-2/10 (d1) 4-5/10 (d2-3) 8/10 (d4) 8/9 (d7) 9/9 (d14)	2-3/10 (h0-6) 3-5/10 (d1-3) 10/10 (d4) 8/9 (d7) 9/9 (d14)	1/10 (h0-6) 0-1/9-10 (d1) 7-8/8 (d2-4) 7-8/8 (d7) 7/7 (d14)	2/2 (d4,7)	0/2 (h0-2) 0/1 (h4-6)

* = number of clinical signs/number of surviving animals, point in time (h = hours, d = day) in parenthesis

C. BODY WEIGHT

For the 2500, 3500, 5000, and 7000 mg/kg body weight dose levels, although some animals lost weight between 7 and 14 days, all surviving animals gained weight throughout the study (see table below).

Table B.6.2.1.39-3: Acute Oral Toxicity Study In Rats [redacted] 1979): Body weight development

Dose level [mg/kg bw]	**2500**	**3500**	**5000**	**7000**	**9900**
	Body weight (g)				
Sex	**Males**				
Day 0					
	268	279	250	290[$]	291[$]
	274	262	245	292[$]	265[$]

Table B.6.2.1.39-3: Acute Oral Toxicity Study In Rats ([redacted] 1979): Body weight development

Dose level [mg/kg bw]	2500	3500	5000	7000	9900
Body weight (g)					
	269	290	252	289$	271$
	281	294	248	294$	294$
	284	262	240	287$	284$
Mean ± SD	**275.2 ± 7.1**	**277.4 ± 15.1**	**247.0 ± 4.7**	**290 ± 2.7**	**281.0 ± 12.6**
Day 7					
	320	326	305	-	-
	307	323	310	-	-
	324	345	321	-	-
	-	369	276	-	-
	339	186	256	-	NR
Mean ± SD	**322.5 ± 13.2**	**309.8 ± 71.6**	**293.6 ± 26.8**		
Termination#					
	365	356	346	+	+
	340	360	352	+	+
	349	379	370	+	+
	201 (Day 5) $	396	331	+	+
	363	177 (Day 8) $	305	+	NR
Mean ± SD	**323.6 ± 69.3**	**333.6 ± 89.0**	**340.8 ± 24.4**		
Sex	**Females**				
Day 0					
	229	240	241	241$	239$
	266	236	246	291$	270$
	279	234	250	236	232$
	284	239	235	243$	236$
	288	249	225	225	260$
Mean ± SD	**269.2 ± 24.0**	**239.6 ± 5.8**	**239.4 ± 9.8**	**247.2 ± 25.5**	**247.4 ± 16.6**
Day 7					
	241	254	-	-	-
	291	253	274	-	-
	310	246	274	259	-
	312	250	-	-	-
	325	259	268	241	-
Mean ± SD	**295.8 ± 32.9**	**252.4 ± 4.8**	**272.0 ± 3.5**	**250.0 ± 12.7**	
Termination#					
	237	256	212 (Day 1) $	+	+
	296	247	285	+	+
	304	242	219 (Day 9) $	258	+
	298	257	200 (Day 2)$	+	+
	323	263	269	236	+
Mean ± SD	**291.6 ± 32.3**	**253.0 ± 8.4**	**237.0 ± 37.6**	**247.0 ± 15.6**	

= Terminal body weight recorded on Day 14 unless otherwise noted in parenthesis, $ = Animal died spontaneously,
+ = No terminal body weights were recorded for animals dying spontaneously on Day 1 of the study. Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively.
NR: not recorded

D. NECROPSY

A summary of the gross necropsy findings for the decedents and the animals necropsied at the conclusion of the 14-day observation period is presented in the table below.

Table B.6.2.1.39-4: Acute Oral Toxicity Study In Rats [redacted] 1979): Summary of Necropsy Findings

Dose Level (mg/kg bw)	Animals Necropsied at 14 Days	Decedents
2500	Discoloured lungs (in 6/9), liver (in 9/9), and/or kidneys (in 9/9)	Urinary and faecal staining of the abdomen (1/1) Discoloured lungs (1/1) Fluid filled stomach (1/1) Fluid filled and/or distended intestines (1/1)
3500	Discoloured lungs (9/9), liver (9/9), and/or kidneys (9/9) Air filled intestines (1/9)	Discoloured lungs (1/1)
5000	No observations (7/7)	Oral and/or nasal discharge (2/3) Urinary and/or faecal staining of the abdomen (3/3) Discoloured lungs (2/3) and/or liver (3/3) Fluid filled and/or discoloured stomach and/or intestines (3/3)
7000	Discoloured lungs (1/2), liver (2/2), and/or kidneys (1/2) Air filled intestines (1/2)	Oral discharge (3/8) Fluid filled intestines and/or stomach (7/8) Discoloured liver (1/8), and/or kidneys (8/8) Urinary and/or faecal staining of the abdomen (2/8)
9900	Not applicable	Discoloured lungs (3/10), liver (2/10), and/or kidneys (7/10) Fluid filled intestines and/or stomach (9/9) Oral and/or nasal discharge (7/9) Urinary staining of the abdomen (1/9)

III. CONCLUSIONS

The oral LD_{50} of the test material (glyphosate) in rats was calculated to be 5600 mg/kg body weight with 95 % confidence limits of 4900 to 6300 mg/kg body weight.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is considered supplementary only as the study was conducted prior to GLP and not according to any test Guideline and additionally some reporting deficiencies are apparent.
Nevertheless, the outcome of the study can be reported as valid. The acute oral LD_{50} is >5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
The study is considered acceptable (reliable with restrictions) due to the deviations noted above by the applicant. It is agreed, however, that the acute oral LD_{50} of glyphosate in male and female rats was determined to be >5000 mg/kg bw in this study (5600 mg/kg bw; 95% CI: 4900-6300 mg/kg bw).

B.6.2.2. Dermal

B.6.2.2.1. Study 1

Data point	CA 5.2.2/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendment 1
Report No	10/218-002P
Document No	Not reported

Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC 440/2008 B.3 (2008)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of animals was not reported. After treatment the first observation was performed after 1 hour instead of during the first 30 minutes.
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 to be acceptable.

In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application to a shaved area of the back (approximately 10 % area of the total body surface). The application period was 24-hours, followed by a 14-day observation period.

Clinical observations along with a check of viability and mortality were performed on all animals at 1 and 5 hours after dosing and daily for 14 days thereafter. Body weight was measured prior to dosing on Day 0 and on Days 7 and 14. Rats were killed and given a gross macroscopic examination at the end of the 2-week observation period (Day 14).

No mortality occurred. No adverse clinical signs were observed after treatment with the test item or during the 14-day observation period. The body weights were within the range commonly recorded for this strain and age. There was no treatment related macroscopic findings observed in any animals.

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female RjHan:(WI) Wistar rats, observed over a period of 14 days was

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Dry white powder
Lot/Batch #:	569753
Purity:	96.3% w/w glyphosate technical
Stability of test compound:	Stable under storage conditions (< 30 °C), recertification date: 31 August 2011

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rat
Strain:	RjHan:(WI) Wistar
Source:	[redacted]
Sex:	Males and females (nulliparous and nonpregnant)
Age:	Young adults
Weight at dosing:	Males: 228 – 259 g; females: 220 – 231 g
Acclimatisation period:	6 days
Diet:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Type II polypropylene / polycarbonate cages with Lignocel bedding for laboratory animals
Environmental conditions:	Temperature: 20.7 – 24.0 °C
	Humidity: 39 – 65%
	Air changes: 15 – 20 air changes per hour
	Photoperiod: 12-hour light / dark cycle (light 6:00 AM to 6:00 PM)

B: Study design and methods

In life dates: 2010-10-06 to 2010-10-20 (Start of treatment to final sacrifice)

Animal assignment and treatment:
A group of one male and one female and a second group of four male and four female RjHan:(WI) Wistar rats were treated with a single administration of glyphosate technical (96.3 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application. The application period was 24-hours, followed by a 14-day observation period.

The backs of the animals were shaved (approximately 10 % area of the total body surface) approximately 24-hours prior to the treatment. Only those animals without injury or irritation on the skin were used in the test. On Day 0, the test item was moistened with water and applied uniformly over the skin. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24-hours. At the end of the 24-hour exposure period, residual test item was removed, using body temperature water.

A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter. The body weight of all animals was recorded on Day 0 (beginning of the experiment) and on Days 7 and 14. All animals were anaesthetised with Euthasol® 40 % and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. Any gross macroscopic findings were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

There were no clinical signs noted in any animals throughout the study. No treatment related skin irritation was observed in any animal throughout the study.

C. BODY WEIGHT

There were no effects on body weight and body weight gain during the observation period (see table below).

Table 6.2.2.1-1 Glyphosate technical: Acute Dermal Toxicity study in Rats [redacted] 2011): Body weight and body weight gain

Dose level	**5000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 - 14**	**0 - 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
1946 (1951)	228	262	304	225	230	248	76	23
1947 (1952)	259	306	357	225	252	259	98	34
1948 (1953)	240	284	337	220	238	243	97	23
1949 (1954)	244	296	337	231	243	258	93	27
1950 (1955)	238	276	319	220	245	266	81	46
Mean	**241.8**	**284.8**	**330.8**	**224.2**	**241.6**	**254.8**	**89.0**	**30.6**
± SD	**± 11.3**	**± 17.1**	**± 20.1**	**± 4.5**	**± 8.2**	**± 9.2**	**± 9.9**	**± 9.7**

§ = animal No. of males and (females)

D. NECROPSY

No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 5000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.2. Study 2

Data point	CA 5.2.2/002
Report author	[redacted]
Report year	2010
Report title	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats
Report No	24876
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC method B.3. (92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of male animals (7 weeks) was outside of the range specified in the guideline (at least 8 – 10 weeks). Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no signs of toxicity or mortality were reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification: Glyphosate technical grade
Description: White solid powder
Lot/Batch #: 2009051501
Purity: 96.4%
Stability of test compound: Expiry date: 2011-05-15

2. **Vehicle and/ or positive control:** *Aqua ad iniectabilia* [water for injection]
3. **Test animals:**

Species: Rat

Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks; females: approx. 9 weeks
Sex:	Males and females
Weight at dosing:	Males: 233 – 249 g; females: 211 – 229 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, Germany), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in Makrolon cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 40 – 70%
	Air changes: Not reported
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-10-29 to 2009-11-12 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once as a suspension in *aqua ad iniectabilia* for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The administration volume was 10 mL/kg bw. The test substance was applied to 8 layers of gauze. The gauze was covered with a plastic sheet and secured with adhesive plaster on the application site. At the end of the exposure period no residual test item had to be removed. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see Table below).

Table 6.2.2.2-1 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats (2010): Body weight and body weight gain

Dose level	**2000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [%]**	
1 M (6 F)	243	311	349	212	251	256	43.6	20.8
2 M (7 F)	236	309	353	226	265	282	49.6	24.8
3 M (8 F)	233	288	321	211	242	251	37.8	19.0
4 M (9 F)	246	318	359	212	245	249	45.9	17.5
5 M (10 F)	249	329	381	229	269	287	53.0	25.3
Mean	**241.4**	**311.0**	**352.6**	**218.0**	**254.4**	**265.0**	**46.0**	**21.5**
± SD	**6.7**	**15.0**	**21.6**	**8.7**	**12.0**	**18.1**	**-**	**-**

§ = animal No. of males and (females); - = not calculated in report

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute dermal LD_{50} is above 2000 mg/kg bw.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.2.3. Study 3

Data point	CA 5.2.2/003
Report author	[redacted]
Report year	2010
Report title	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats
Report No	24604
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC method B.3. (92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of male animals (7 weeks) was outside of the range specified in the guideline (at least 8 - 10 weeks). Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no signs of toxicity or mortality were reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study, and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate technical grade
Description: White solid powder
Lot/Batch #: 20090506
Purity: 97.3%
Stability of test compound: Expiry date: May 2011

2. **Vehicle and/ or positive control:** *Aqua ad iniectabilia* [water for injection]
3. **Test animals:**

Species:	Rat
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks; females: approx. 9 weeks
Sex:	Males and females
Weight at dosing:	Males: 278 – 292 g; females: 202 – 225 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, Germany), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in Makrolon cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 40 – 70%
	Air changes: Not reported
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-11-12 to 2009-11-26 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once as a suspension in *aqua ad iniectabilia* for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The administration volume was 10 mL/kg bw. The test substance was applied to 8 layers of gauze. The gauze was covered with a plastic sheet and secured with adhesive plaster on the application site. At the end of the exposure period no residual test item had to be removed. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance (see Table below).

Table 6.2.2.3-1 Acute Dermal Toxicity Study of Glyphosate TC in CD Rats [redacted] 2010): Body weight and body weight gain

Dose level	**2000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [%]**	
1 M (6 F)	291	358	384	202	223	237	32.0	17.3
2 M (7 F)	278	331	346	215	242	262	24.5	21.9
3 M (8 F)	281	347	374	208	228	229	33.1	10.1
4 M (9 F)	282	353	371	217	239	252	31.6	16.1
5 M (10 F)	292	367	390	225	249	256	33.6	13.8
Mean	**284.8**	**351.2**	**373.0**	**213.4**	**236.2**	**247.2**	**30.9**	**15.8**
± SD	**6.3**	**13.5**	**16.9**	**8.8**	**10.6**	**13.7**	**-**	**-**

§ = animal No. of males and (females); - = not calculated

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute dermal LD_{50} is above 2000 mg/kg bw.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.2.4. Study 4

Data point	CA 5.2.2/004
Report author	[redacted]
Report year	2009
Report title	Glyphosate: Acute Dermal Toxicity Study in Rats

Report No	12171-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1200 (1998)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Humidity was in the range of 30 – 86 % instead of 30 – 70 %. Weight of two female animals was outside of the range specified in the guideline (200 – 300 g).
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 to be acceptable

The test substance, glyphosate technical, was evaluated for its dermal toxicity potential and relative skin irritancy when a single dose of 5050 mg/kg bw was applied to the intact skin of albino rats. No mortality occurred during the study, and no clinical signs or skin reactions were observed. There was no effect on body weight gain in animals surviving to termination, with the exception of two animals that lost or failed to gain weight during Day 7 – 14 of the study. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 5050 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.71 % (analysis dated 2009-01-08); 96.40 % (analysis dated 2008-10-17)
Stability of test compound:	No data given in the report; however, since the purity of the test material analysed after the test was conducted is consistent with the purity before the test was conducted, the test material is believed to be stable for the duration of the test.

2. Vehicle and/or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Approx. 10 weeks
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 299 – 348 g; females: 189 – 207 g
Acclimation period:	5 days
Diet/Food:	Formulab #5008 (PMI Feeds Inc.), *ad libitum*

Water:	Tap water, *ad libitum*	
Housing:	Individual housing in suspended, wire bottom, stainless steel cages	
Environmental conditions:	Temperature:	19 – 22 °C
	Humidity:	30 – 86%
	Air changes:	10 – 12 / hour
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2008-12-04 to 2008-12-18 (Start of treatment to final sacrifice)

Animal assignment and treatment:

In a limit test, one dose level of 5050 mg/kg bw was examined using five male and five female rats. The test substance was moistened with water and applied once for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of the total body surface). The area of application was covered with surgical gauze patch and secured with non-irritating adhesive tape. The trunk of each animal was then wrapped with vet wrap that was secured in place with non-irritating adhesive tape to prevent possible ingestion of the test substance. After 24-hours the tape was removed, and the skin was washed with water and a cloth to remove excess test material. Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14. Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings, and on Days 4, 7, 11 and 14. On Day 14 after dosing, animals were sacrificed, subjected to gross necropsy, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 5050 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance, with the exception of two animals that lost or failed to gain weight during Day 7 – 14 of the study. A summary of the body weights is given in the table below.

Table 6.2.2.4-1 Glyphosate: Acute Dermal Toxicity Study in Rats [redacted] 2009): Body weight and body weight gain

Dose level	**5050 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 - 14**	**0 - 14**
Animal No.[§]	**Body weight [g]**						**Body weight gain [g]**	
1 M (6 F)	348	356	379	190	204	245	31	55
2 M (7 F)	340	355	387	207	215	217	47	10
3 M (8 F)	319	327	345	189	201	203	26	14
4 M (9 F)	299	318	318	205	218	220	19	15
5 M (10 F)	310	327	340	206	222	219	30	13

Table 6.2.2.4-1 Glyphosate: Acute Dermal Toxicity Study in Rats [redacted] 2009): Body weight and body weight gain

Mean	**323.2**	**336.6**	**353.8**	**199.4**	**212.0**	**220.8**	**30.6**	**21.4**
± SD	**± 20.5**	**± 17.6**	**± 28.7**	**± 9.1**	**± 9.1**	**± 15.2**	**± 10.3**	**± 18.9**

§ = animal No. of males and (females)
Note: Mean and standard deviation as well as body weight gain were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 5050 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5050 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 5050 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.5. Study 5

Data point	CA 5.2.2/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Acute dermal toxicity study in rats
Report No	C22875
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), Commission Regulation (EC) No 440/2008 (2008) method B.3
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Weight of female animals (189.8 – 208.6 g) was outside of the range specified in the guideline (200 – 300 g). Since the body weights are only slightly outside the suggested weight range, the deviation is not considered to impact the outcome of the study.
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 to be acceptable.

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential when applied dermally to an area of clipped skin of male and female HanRcc: WIST (SPF) rats at a dose level of 2000 mg/kg bw. No mortality occurred during the study. No clinical signs were observed during the course of the study. No local dermal signs were observed in any of the treated male animals. In four female animals, slight erythema, scaling, and scabs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was calculated to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66% Expiry date: July 2010
Stability of test compound:	Stable under storage conditions (20 ± 5 °C, light protected). Stable in purified water for 2 days.

2. Vehicle and/or positive control: Purified (deionised, PURLAB Option-R unit-treated) water

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	[redacted]
Age:	Males: 9 weeks; females: 11 weeks
Sex:	Males and females
Weight at dosing:	Males: 238.9 – 258.3 g; females 189.8 – 208.6 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 61/08 (Provimi Kliba AG, 4303 Kaiseraugst / Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, 4132 Muttenz / Switzerland) during treatment and observation period.
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 30 – 70 %
	Air changes: 10 – 15 / hour
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-01-20 to 2009-02-03 (Start of treatment to final sacrifice)

Animal assignment and treatment:

Single dose of 2000 mg/kg bw of test substance (glyphosate technical) was applied dermally to an area of clipped skin (approximately 10 % of total body surface area) of five male and five female young adult rats. The treatment area was covered with a semi-occlusive dressing. Application volume was 4 mL/kg bw. Twenty-four hours after the application the dressing was removed and the skin was flushed with lukewarm tap water and dapped off with disposable paper towels. All animals were re-shaven on test days 4 and 9 to facilitate the reading of the local reactions. The animals were evaluated for effects on the day of dosing (Day 0) at 30 minutes and at 1, 2, 3 and 5 hours after application and once daily during Days 1 – 14. Clinical observations, dermal findings, body weights and gross post mortem examinations were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. CLINICAL OBSERVATIONS

No systemic clinical signs were observed during the course of the study.

C. BODY WEIGHT

A slight body weight loss (0.3 – 0.8 %) was observed in two females between acclimatisation and treatment start. The animals recovered until the end of the study. In spite of this body weight loss, the body weights of all animals were considered to be within the range commonly recorded for this strain and age. A summary of the body weights is given in the table below.

Table 6.2.2.5-1 Glyphosate Technical: Acute Dermal Toxicity Study in Rats [redacted] 2009): Body weight and body weight gain

Dose level	**2000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
1 M (6 F)	250.9	268.6	297.6	203.5	201.8	215.5	46.7	12.0
2 M (7 F)	238.9	264.6	289.7	200.4	205.6	214.5	50.8	14.1
3 M (8 F)	253.6	270.7	301.9	189.8	194.0	205.0	48.3	15.2
4 M (9 F)	252.4	281.5	311.5	195.8	195.2	203.5	59.1	7.7
5 M (10 F)	258.3	288.5	318.0	208.6	213.0	223.0	59.7	14.4
Mean	**250.8**	**274.8**	**303.7**	**199.6**	**201.9**	**212.3**	**52.9**	**12.7**
± SD	**± 7.2**	**± 9.9**	**± 11.2**	**± 7.2**	**± 7.8**	**± 8.1**	**± 6.1**	**± 3.0**

§ = animal No. of males and (females)

Note: Body weight gain and mean and SD body weight gain were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No macroscopic findings were recorded at necropsy.

E. SKIN OBSERVATIONS

No local dermal signs were observed in any of the treated male animals. After removal of the patch, a very slight erythema was noted in four females on Day 4 and persisted up to Days 6, 11 or 12. Scaling was observed in the same four females on Day 4 and persisted up to Days 10, 11 and 12. Scabs were recorded in two females on Day 9 that persisted to Day 11 (see table below).

Table 6.2.2.5-2 Glyphosate Technical: Acute Dermal Toxicity Study in Rats [redacted] 2009): Skin observation data

Dose level		**2000 mg/kg bw**										
Day		**0* – 2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12 – 14**
Sex	**Skin observations**											
M	Erythema	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scaling	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scabs	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
F	Erythema	(0/5)	(0/5)	1 (4/5)	1 (4/5)	1 (4/5)	1 (3/5)	1 (3/5)	1 (3/5)	1 (3/5)	1 (3/5)	1 (1/5)
	Scaling	(0/5)	(0/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (4/5)	√ (3/5)	√ (3/5)	√ (2/5)
	Scabs	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	√ (0/5)	√ (2/5)	√ (2/5)	(2/5)	(0/5)

1 = slight erythema, √ = scaling or scabs noted.

* Examinations on Day 0 were performed within the first 30 minutes and 1, 2, 3, and 5 hours after exposure.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 2000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.6. Study 6

Data point	CA 5.2.2/006
Report author	[redacted]
Report year	2009
Report title	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats
Report No	23912
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), EC method B.3. (92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of male animals (7 weeks) was outside of the range specified in the guideline (at least 8 – 10 weeks). Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no signs of toxicity or mortality were reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study, and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical grade
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8%
Stability of test compound:	Expiry date: 2010-08-01

2. **Vehicle and/ or positive control:** *Aqua ad iniectabilia* [water for injection]
3. **Test animals:**

Species:	Rat
Strain:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: 51 days; females: 65 days
Sex:	Males and females
Weight at dosing:	Males: 224 – 234 g; females: 200 – 217 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, Germany), *ad libitum* except for approx. 16 h before administration
Water:	Tap water, *ad libitum*
Housing:	Individual housing in Makrolon cages (type III plus) with granulated textured wood as bedding material
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 40 – 70%
	Air changes: 12 – 18 / hour
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-02-18 to 2009-03-04 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was applied once as a suspension in *aqua ad iniectabilia* for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of total body surface). The administration volume was 10 mL/kg bw. The test substance was applied to 8 layers of gauze. The gauze was covered with a plastic sheet and secured with adhesive plaster on the application site. At the end of the exposure period no residual test item had to be removed. Observations for mortality and clinical/behavioural signs of toxicity were made before, immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24-hours after administration and at least once daily thereafter for 14 days. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. The skin was observed for the development of erythema and oedema. At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically, and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. The acute dermal LD_{50} is above 2000 mg/kg bw.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.2.7. Study 7

Data point	CA 5.2.2/007
Report author	[redacted]
Report year	2008
Report title	Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical
Report No	[redacted]3996.310.456.07
Document No	Not reported
Guidelines followed in study	OECD 402 (1987)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. One female rat weighed less than 200 g on the day of test item application.
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 to be acceptable.

The test substance, glyphosate technical, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study, and no clinical signs or skin reactions were observed. All animals gained the expected body weight, except for two females on the second week of the observation period. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05%
Stability of test compound:	No data given in the report

2. **Vehicle and/or positive control:** Deionised water
3. **Test animals:**

Species:	Rat
Strain:	Wistar Hannover
Source:	[redacted]
Age:	9 – 11 weeks

Sex:	Males and females (nulliparous and non-pregnant)	
Weight at dosing:	Males: 266 – 298 g; females: 199 – 213 g	
Acclimation period:	7 days	
Diet/Food:	Autoclaved Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda., Curitiba - PR, Brazil), *ad libitum*	
Water:	Filtered drinking water, *ad libitum*	
Housing:	Individual housing in polypropylene rodents cages with wire mesh tops and bedding material	
Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	30 – 70%
	Air changes:	At least 10 / hour
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2007-09-11 to 2008-06-11

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 2000 mg/kg bw was moistened with deionised water and applied once for 24-hours on the shaved intact dorsal skin of the rats (approximately 10 % of the total body surface). The test item was held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site was further covered using adhesive tape (semi-occlusive dressing). At the end of the exposure period the dressing was removed, and residual test substance was wiped off with deionised water. Observations for mortality and clinical/behavioural signs of toxicity were made once within the first 30 minutes after dosing, three times more during the first 4 hours after dosing, and daily thereafter for a period of 14 days. Individual body weights were determined before the application of the test item (Day 0) and on Days 7 and 14. On Day 14 after dosing, each animal was sacrificed. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 2000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

All animals gained the expected body weight, except for two females in the second week of the observation. A summary of the body weights is given in the table below.

Table 6.2.2.6-1 Acute Dermal Toxicity Study in Wistar Hannover Rats for Glyphosate Technical [redacted] 2008): Body weight and body weight gain

Dose level	**2000 mg/kg bw**								
Sex	**Males**			**Females**			**Males**	**Females**	
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**7 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [%]**		
49 M (56 F)	289	309	326	203	204	213	37	9	10
50 M (57 F)	266	279	293	199	204	211	27	7	12
51 M (58 F)	298	319	343	213	216	214	45	-2	1
53 M (59 F)	297	321	339	202	207	210	42	3	8
54 M (60 F)	298	322	346	211	219	219	48	0	8
Mean	**289.6**	**310.0**	**329.4**	**205.6**	**210.0**	**213.4**	**39.80**	**3.40**	**7.80**
± SD	**± 13.72**	**± 18.08**	**± 21.73**	**± 6.07**	**± 7.04**	**± 3.51**	**± 8.23**	**± 4.62**	**± 4.15**

§ = animal No. of males and (females)

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD50 is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 2000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.8. Study 8

Data point	CA 5.2.2/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Acute dermal toxicity study in rats
Report No	B02283
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), EC method B.3. (92/69/EEC), JMAFF, Guidelines for Preparation of Study Results, Acute Dermal Toxicity Studies, Guideline 2-1-2. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260,

	on 16 March 2005; English translation by AGIS on 17 Oct 2005
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Body weight of two female animals (194.8 and 199.9 g) was outside of the range specified in the guideline (200 – 300 g).
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 to be acceptable.

Glyphosate technical (NUP 05068) diluted in vehicle (PEG 300) was applied dermally at 2000 mg/kg bw (with an application volume of 6 mL/kg bw) to five male and five female rats. The application period was 24-hours. The animals were examined daily for mortality, viability, and clinical signs. Body weights were recorded on Days 0 (prior to administration), 7, and 14. No deaths occurred during the study. No clinical signs nor body weight changes were observed during the course of the study. No macroscopic findings were observed at necropsy. The Glyphosate Technical (NUP 05068) dermal LD_{50} (rat) is

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1%
Stability of test compound:	Stable under storage conditions.

2. Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	Males: 8 weeks; females: 11 weeks
Sex:	Males and females
Weight at dosing:	Males: 233.0 – 260.2 g; females: 194.8 – 216.4 g
Acclimation period:	6 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz, Switzerland)

Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	30 – 70%
	Air changes:	10 – 15 / hour
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2006-12-19 to 2007-01-02 (Start of treatment to final sacrifice)

Animal assignment and treatment:

One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10 % of the total body surface. Only those animals without injury or irritation on the skin were used in the test. On Day 0, the test item was applied at a dose of 2000 mg/kg bw with an application volume of 6 mL/kg bw in vehicle (PEG 300) on the intact skin and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. Twenty-four hours after the application, the dressing was removed, and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Animals were examined for clinical signs at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on Day 0 and once daily during Days 1 – 14. Local signs were noted once daily from Day 1 – 14. Mortality was recorded at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on Day 0 and twice daily during Days 1 – 14. Body weights were recorded on Day 0 prior to administration and on Days 7 and 14. The fur of all animals was shaved on Days 3 and 8 just after the assessment of the reaction to facilitate the skin reading for the next day. All animals were sacrificed at the end of the observation and examined macroscopically.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No systemic or local signs of toxicity were observed during the study period.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of the test material (glyphosate technical) after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 2000

mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.9. Study 9

Data point	CA 5.2.2/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate technical material: Acute dermal toxicity study in rats
Report No	B02766
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), JMAFF 12 NohSan 8147 (2000)
Deviations from current test guideline (OECD 402, 2017)	A group of one rat per sex and a second group of four rats per sex were treated in a sequential manner, instead of two animals, preferred females, per dose. Weight of three female animals was outside of the range specified in the guideline (200 – 300 g).
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 to be acceptable.

In an acute dermal toxicity study, a group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1 % w/w glyphosate technical) at 5000 mg/kg bw by dermal application. The test item was moistened with purified water before application. The application period was 24-hours.

The animals were examined daily during the acclimatization period and mortality, viability, and clinical signs were recorded. All animals were examined for clinical signs once at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on Day 0 and once daily during Days 1 to 14. Local signs were noted once daily from Day 1 to 14. Mortality/viability was recorded once at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on Day 0 (with the clinical signs) and twice daily during Days 1 – 14. Body weights were recorded on Day 0 (prior to administration) and on Days 7 and 14. All animals were necropsied and examined macroscopically.

No deaths occurred and no systemic signs or local signs of irritation were noted during the course of the study. The body weights of the animals were within the range commonly recorded for this strain and age. No macroscopic findings were observed at necropsy.

The acute dermal LD_{50} of glyphosate technical material after a single dermal administration to male and female HanRcc:WIST (SPF) rats, observed over a period of 14 days was

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Glyphosate technical material
Description:	White powder
Lot/Batch number:	0507
Purity:	96.1% w/w
Stability of test compound:	Re-analysis date August 2008. Stable under storage conditions (room temperature range 20±5 °C, protected from light and humidity).
2. Vehicle and/ or positive control:	Purified water
3. Test animals:	
Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Sex:	Males and females
Age:	Males: 8 weeks; females: 11 weeks
Weight at dosing:	Males: 222.7 – 247.0 g; females: 191.3 – 204.2 g
Acclimation period:	7 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland), *ad libitum*
Water:	Community tap water, *ad libitum*
Housing:	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz, Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70% Air changes: 10 – 15 air changes per hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2006-12-07 to 2006-12-22 (Start of treatment to final sacrifice)
Animal assignment and treatment:

A group of one male and one female and a second group of four male and four female HanRcc:WIST (SPF) rats were treated with glyphosate technical material (96.1% w/w glyphosate technical) at 5000 mg/kg bw by dermal application. One day before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10% of the total body surface. Only those animals without injury or irritation on the skin were used in the test. The test item was moistened with purified water before application (0.5 – 0.6 mL). The dry paste was applied evenly on the intact skin of the clipped area and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and anchored with tape. The area of skin covered by the test item was approximately 8 cm^2 for the males and females. Twenty-four hours after application the dressing was removed, and the skin was flushed with lukewarm tap water and dried with disposable paper towels. Thereafter, the reaction sites were assessed.

A single animal of each sex was treated first. As no deaths, severe local effects, or severe systemic symptoms were observed after the 24-hour exposure, the test was completed using the four remaining male and female animals for an exposure period of 24-hours.

The fur of all males and females was shaved, on Day 5 (female no. 2), on Days 4 and 8 (male no. 1), on Days 3 and 7 (males nos. 3 – 6 and females nos. 7 – 10) just after the assessment of the reaction to facilitate the skin reading for the next day. The animals were checked daily for mortality/viability during the acclimatization period, at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on Day 0 (with the clinical signs) and twice daily during Days 1 to 14. Clinical observations were recorded daily during the acclimatisation period,

at approximately 30 minutes, 1, 2, 3 and 5 hours after administration on Day 0 and once daily during Days 1 – 14. The animals were examined daily for local signs at the application site. Body weights were recorded on Days 0 (prior to administration), 7, and 14. All animals were sacrificed at the end of the observation period and macroscopic examinations were performed. No organs or tissues were retained.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No systemic signs or local signs of irritation were noted during the course of the study.

C. BODY WEIGHT
The body weights of the animals were within the range commonly recorded for this strain and age.

D. NECROPSY
No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD50 is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 5000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.10. Study 10

Data point	CA 5.2.2/010
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test
Report No	15275
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA OPPTS 870.1200 (1998), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Weight of female animals (193 – 200 g) was outside of the range specified in the guideline (200 – 300 g). Humidity and air changes were not reported. The deviations are not considered to impact the outcome of the study.
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 to be acceptable.

The test substance, glyphosate acid technical, was evaluated for its acute dermal toxicity potential in rats when administered as single dose of 5000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23%
Stability of test compound:	Test substance was expected to be stable for the duration of testing.

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[redacted]
Age:	8 weeks
Sex:	Males and females
Weight at dosing:	Males: 231 – 264 g; females: 193 – 200 g
Acclimation period:	8 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 19 – 23 °C Humidity: Not reported Air changes: Not reported Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2004-05-05 to 2004-05-19

Animal assignment and treatment:

One dose level group of five male and five female rats was examined in a limit test. The dose level of 5000 mg/kg bw was applied once as a dry paste (70% w/w mixture in distilled water) for 24-hours on the shaved intact dorsal skin of the rats (approximately 10% of total body surface). The test substance was held in contact with the skin with a gauze pad and Durapore tape. After 24-hours exposure the pads were removed and the test sites were gently cleansed of any residual test substance. Observations for mortality and clinical/behavioural signs of toxicity were made during the first several hours post-dosing and at least once daily thereafter for 14 days after dosing. Individual body weights were recorded just prior to dosing and on Days 7 and 14. On Day 14 after dosing, each animal was sacrificed. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

A single dermal administration of 5000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate acid technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 5000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.11. Study 11

Data point	CA 5.2.2/011
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Acute Dermal Toxicity in the Rat
Report No	[redacted]P/4664
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA 81-2, EC method B.3. (92/69/EEC, 1992)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Specific age of animals was not reported, but it was stated that young adult rats were used. No observations during the first 30 minutes after dosing were made, but only once between one four hours after dosing. Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no mortality and only limited skin irritation was reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations 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 to be acceptable.

In an acute dermal toxicity study, a group of five male and five female, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid in deionised water and observed for 14 days.

No mortality occurred during the study. There were no significant signs of systemic toxicity and practically no signs of skin irritation. One male showed slight erythema on Days 1 and 2, and one female had small scabs from Day 2 to 7. Most animals had exceeded their initial weight by the end of the study. There were no treatment-related findings at examination *post mortem*.

The acute dermal LD_{50} of glyphosate acid was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch number:	P24
Purity:	95.6% w/w
Stability of test compound:	The test substance was used within the expiry date.

2. Vehicle and/ or positive control:	Deionised water
3. Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	[redacted]
Sex:	Males and females
Age:	Young adult
Weight at dosing:	Males: 250 – 274 g; females: 203 – 216 g
Acclimation period:	At least 6 days
Diet/Food:	Diet (PCD), supplied by Special Diet Services Limited, Witham, Essex, UK, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.
Environmental conditions:	Temperature: 21 ± 2 °C Humidity: 40 – 70% Air changes: Approximately 25 – 30 / hour Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1995-03-16 to 1996-03-30

Animal assignment and treatment:

A group of five male and five female, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid. Sixteen to 32 hours before application, the hair was removed by clipping from an area on the dorso-lumbar region of each rat (approximately 10 cm × 5 cm). The appropriate amount of test substance was moistened to a dry paste with 0.6 - 0.8 mL of deionised water. On the day of treatment (Day 0), approximately half the application site was covered by test substance (equivalent to 20.0 – 21.9 mg/cm^2 for males and 16.2 – 17.3 mg/cm^2 for females). The application site was covered with a 4-ply gauze patch (approximately 7 cm × 7 cm) and kept in place for 24-hours using an occlusive dressing. The gauze patch was covered by a piece of plastic film (7 cm × 7 cm), held in place using adhesive bandage (25 cm × 7.5 cm) secured by two pieces of PVC tape. At the end of the 24-hour contact period, the dressings were carefully removed and the skin cleansed of any residual test substance using clean warm water.

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. The animals were observed for signs of systemic toxicity once between one and four hours of dosing. Subsequent observations were made daily, up to Day 14.

The animals were weighed immediately before dosing (Day 0) and on Days 2, 4, 7 and 14. All animals were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

There were no significant signs of systemic toxicity (only urinary incontinence in three of five females due to bandaging, which is not considered to be of toxicological significance). The skin of all animals was stained cream by the test substance, but this did not prevent the assessment of erythema. There were practically no signs of skin irritation. One male showed slight erythema on Days 1 and 2, and one female had small scabs from Day 2 to 7 (see table below).

Table 6.2.2.11-1 Glyphosate Acid: Acute Dermal Toxicity study in the rat [redacted] 1996): Skin observation data

Dose level		**2000 mg/kg bw**								
Day		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8 – 14**
Sex	**Skin observations**									
M	Skin stained	(0/5)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	(5/5)	(0/5)	(0/5)
	Erythema	(0/5)	1 (1/5)	1 (1/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scabs	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
F	Skin stained	(0/5)	(5/5)	(5/5)	(5/5)	(1/5)	(1/5)	(1/5)	(0/5)	(0/5)
	Erythema	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)
	Scabs	(0/5)	(0/5)	2 (1/5)	2 (1/5)	2 (1/5)	2 (1/5)	2 (1/5)	2 (1/5)	(0/5)
	Signs of urinary incontenence	(0/5)	(3/5)	(3/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)

1 = slight erythema, 2 = small scattered.

C. BODY WEIGHT

Two males and three females lost weight initially, but all had exceeded their initial weight by Day 4, and except for one female, continued to gain weight throughout the remainder of the study. One female lost weight slightly from Day 4. A summary of the body weights is given in the table below.

Table 6.2.2.11-2 Glyphosate Acid: Acute Dermal Toxicity study in the rat [redacted] 1996): Body weight and body weight gain

Dose level	**2000 mg/kg bw**											
Sex	**Males**					**Females**					**Males**	**Females**
Day	**0**	**2**	**4**	**7**	**14**	**0**	**2**	**4**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.[§]	**Body weight [g]**										**Body weight gain [g]**	
6 M (51 F)	250	248	274	297	337	205	205	209	233	246	87	41
7 M (52 F)	253	257	282	305	353	216	197	218	217	215	100	-1
8 M (53 F)	274	274	291	314	352	211	214	222	247	258	78	47
9 M (54 F)	262	268	286	306	346	207	205	216	230	236	84	29
10 M (55 F)	256	252	270	286	327	203	201	204	228	232	71	29
Mean	**259.0**	**259.8**	**280.6**	**301.6**	**343.0**	**208.4**	**204.4**	**213.8**	**231.0**	**237.4**	**84.0**	**29.0**

Table 6.2.2.11-2 Glyphosate Acid: Acute Dermal Toxicity study in the rat [redacted] 1996): Body weight and body weight gain

± SD	**± 9.5**	**± 10.9**	**± 8.6**	**± 10.6**	**± 11.0**	**± 5.2**	**± 6.3**	**± 7.2**	**± 10.8**	**± 16.1**	**± 10.8**	**± 18.5**

§ = animal No. of males and (females)
Note: Body weight gain values were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

Red mottled lungs were seen in one male. This is a common spontaneous finding in rats of this age and strain and is considered not to be treatment-related (see table below).

Table 6.2.2.11-3 Glyphosate Acid: Acute Dermal Toxicity study in the rat [redacted] 1996): Necropsy data

Dose level	**2000 mg/kg bw**	
Sex	**Males**	**Females**
Animals examined	5	5
NAD	4	5
Lung		
- mottled: (moderate) red	1[§]	0

NAD = Number of animals without abnormalities
[§] Note that this finding is described as occurring for a female in the text of the report, but indicated for animal No. 10, a male, in Table 4 of the report.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate acid after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Although the test conditions are considered worst-case due to the occlusive dressing used, it is agreed with the conclusion. In the absence of mortality the acute dermal LD_{50} > 2000 mg/kg bw in male and female rats.

B.6.2.2.12. Study 12

Data point	CA 5.2.2/012
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute dermal toxicity study in rats
Report No	94-0154
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA FIFRA Guideline Subdivision F Acute Dermal Toxicity 81-2 (1984), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of rats (7 weeks) and weight (178 – 198 g) of female animals was outside of the range specified in the guideline (at least 8 – 10 weeks and 200 – 300 g, respectively). No observation during the first 30 minutes after dosing. The dressing that was used was not further specified other than a closed patch. It is assumed that this describes an occlusive dressing instead of a semi-occlusive dressing. Since, an occlusive dressing is considered worst-case and no signs of toxicity or mortality were reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations reported. Additionally, a solvent control group was included which is not a requirement.
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 to be acceptable.

Glyphosate technical was singly administered to specific pathogen free SD rats (Crj:CD) in order to investigate its acute dermal toxicity at a dose level of 2000 mg/kg bw using five males and five females. There were no deaths in either sex. Neither clinical signs nor gross abnormalities at necropsy were noted in any animals. No body weight losses were recorded at 7 and 14 days after the administration when compared with the body weights of the day of administration. Based on the results mentioned above, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical (Code HR-001)
Description:	White crystal
Lot/Batch #:	940908-1
Purity:	95.68%
Stability of test compound:	No data given in the report.

2. **Vehicle and/ or positive control:** Deionised water
3. **Test animals:**

Species:	Rat
Strain:	Specific pathogen free SD rats (Crj:CD)
Source:	[redacted]
Age:	7 weeks
Sex:	Males and females
Weight at dosing:	Males: 248 – 268 g; females: 178 – 198 g
Acclimation period:	9 days
Diet/Food:	Certified Pellet Diet MF (Oriental Yeast Co., Tokyo, Japan), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire mesh bottom, stainless steel cages
Environmental conditions:	Temperature: 24.0 - 24.5 °C
	Humidity: 53 – 55%
	Air changes: 12 times/hour
	Photoperiod: 12-hour light/dark cycle (light 7:00 AM to 7:00 PM)

B: Study design and methods

In life dates: 1995-02-09 – 1995-02-23 (Start of treatment to final sacrifice)

Animal assignment and treatment:

The test material was finely ground with a mortal and pestle, moistened with deionised water, and applied onto the shaved skin of five male and five female specific pathogen free SD rats (Crj:CD) at a dose level of 2000 mg/kg bw by a closed patch for 24-hours. A negative control group received 0.5 mL of deionised water by the same procedure. After removal of closed patch, the test substance remaining in contact with the skin site was washed off with lukewarm water. Mortality and clinical signs were recorded 1, 3, and 6 hours after administration and at least once daily thereafter until the termination of the 14-day observation period. All animals were weighed on the day of administration and on Days 7 and 14 after administration. The surviving animals were sacrificed after completion of the observation period (Day 14) and examined for gross abnormalities. All animals were subjected to necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs were noted in any animals of control and 2000 mg/kg bw groups.

C. BODY WEIGHT

All animals gained their body weights on Days 7 and 14 after administration.

D. NECROPSY

There was no macroscopic abnormality in any surviving animals at final necropsy after completion of the observation period.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 2000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.13. Study 13

Data point	CA 5.2.2/013
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD50 tests with [redacted] Glyphosate acid technical in rats, limit test"
Report No	00917
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA 81-2 (1984)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Age of animals was not reported. Body weight of male animals was outside of the range specified in the guideline (200 – 300 g). The number of air changes was not specified. Body weights were only recorded once prior to start of study and not once a week during the study. Time of observation on the day of application was not specified according to the test guideline. Individual animal data were not provided. Instead of a semi-occlusive dressing an occlusive dressing was used.
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 to be acceptable (reliable with restrictions) due to the limitations of the study.

The acute dermal toxicity potential of the test material, glyphosate acid technical (Batch: 1073; Purity: 97.6 %), was investigated in groups of five male and five female Sprague-Dawley rats. The test substance was applied at doses of 2000 mg/kg bw (limit test) to clipped dorsal and ventral skin using cotton seed oil as a vehicle and covered by occlusive dressing for 24-hours. After exposure residual test substance was removed with water and the rats were observed for 14 days.

Mortality and clinical signs of toxicity were monitored on the day of administration and daily thereafter. All animals were subjected to gross necropsy.

No mortality and clinical signs occurred during the study. Gross necropsy revealed splenomegaly and centrilobular hepatic congestion. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid technical
Description:	Fine white granular powder
Lot/Batch #:	1073
Purity:	97.6% (certificate of analysis)
Stability of test compound:	Expiry date: August 1996

2. Vehicle and/or positive control: Cotton seed oil (500 mg/mL)

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	Males: 150.2 – 237.2 g; females: 205.6 – 260.9 g
Acclimation period:	At least 5 days
Diet/Food:	Rodent pelleted feed, *ad libitum*
Water:	Not specified, *ad libitum*
Housing:	Groups of 5/sex in standard rodent polycarbonate cages
Environmental conditions:	Temperature: 19 – 21 °C
	Humidity: 62 – 73%
	Air changes: Not specified
	Photocycle: 12-hour light / dark cycle

B: Study design and methods

Study dates: 1995-02-23 to 1995-03-13

Animal assignment and treatment:

Approximately 24-hours before the application of the test material, the fur was removed by clipping the dorsal and ventral area of five male and five female rats.

The test substance as a dilution in cotton seed oil was administered dermally (approximately 10 % of the total body surface area) in a single application at a dose level of 2000 mg/kg bw (limit test). The treatment volume ranged between 0.73 and 1.10 mL, spread in a thin uniform film. The test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site was further covered by Elastoplast to retain the gauze dressing and to ensure that animals could not ingest the test substance. After 24-hours the treated skin was wiped with water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made immediately (1 – 2 hours) after treatment, and thereafter at least once a day. The animals were sacrificed at the end of the 14-day observation period and subjected to a gross necropsy.

II. RESULTS AND DISUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed during the 14–day observation period.

C. BODY WEIGHT
Body weights were not reported.

D. NECROPSY
Splenomegaly and centrilobular hepatic congestion were observed in male and female animals.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate acid technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except to minor deviations, the study is in accordance to the OECD 402 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Due to the limitations (minimal reporting and occlusive dressing) the study is considered acceptable (reliable with restrictions). Under the study conditions the acute dermal LD_{50} > 2000 mg/kg bw in males and female rats.

B.6.2.2.14. Study 14

Data point	CA 5.2.2/014
Report author	[redacted]
Report year	1995
Report title	Final report for "Oral and dermal LD_{50} tests with [redacted] Glyphosate 62 % IPA in rats, limit test"
Report No	00926
Document No	Not reported
Guidelines followed in study	OECD 402 (1987), US EPA 81-2 (1984)
Deviations from current test	No stepwise approach was used, five rats of both sexes instead of two

guideline (OECD 402, 2017)	animals, preferred females, were used per dose. Age of animals was not reported. The number of air changes was not specified. Body weight of male and female animals was outside of the range specified in the guideline (200 – 300 g). Body weights were only recorded once prior to start of study and not once a week during the study. Time of observation on the day of application was not specified according to the test guideline. Individual animal data were not provided. Instead of a semi-occlusive dressing an occlusive dressing was used.
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 to be acceptable (reliable with restrictions) due to the limitations of the study.

The acute dermal toxicity potential of the test material, glyphosate salt (Batch: 940950; Purity: 61.8 %), was investigated in five male and five female Sprague-Dawley rats. The test substance was applied undiluted to clipped dorsal and ventral skin at doses of 2000 mg/kg bw (limit test) and covered by occlusive dressing for 24-hours. After exposure the test substance was removed using water and the rats were observed for 14 days.

Mortality and clinical signs of toxicity were monitored on the day of administration and daily thereafter. All animals were subjected to gross necropsy.

No mortality and clinical symptoms occurred during the study. The gross necropsy revealed severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion, and subcapsular renal petechiae in all animals. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate 62% IPA
Description:	Light greenish viscous liquid
Lot/Batch #:	940950
Purity:	61.8 %
Stability of test compound:	Expiry date: August 1996

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	Male: 153.0 – 212.2 g; female: 197.2 – 248.7 g

Acclimation period:	At least 5 days	
Diet/Food:	Pelleted feed, *ad libitum*	
Water:	Not specified, *ad libitum*	
Housing:	Groups of 5/sex in standard rodent polycarbonate cages	
Environmental conditions:	Temperature:	19 – 21 °C
	Humidity:	62 – 73 %
	Air changes:	Not specified
	Photocycle:	12-hour light / dark cycle

B: Study design and methods

Study dates: 1995-02-23 to 1995-03-13

Animal assignment and treatment:

Approximately 24-hours before the application of the test material, the fur was removed by clipping the dorsal and ventral area of five male and five female rats.

The undiluted test substance was administered dermally (approximately 10 % of the total body surface area) in a single application at a dose level of 2000 mg/kg bw. The treatment volume ranged between 0.59 and 0.78 mL. The test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape. The test site was further covered by Elastoplast to retain the gauze dressing and to ensure that animals could not ingest the test substance. After 24-hours the treated skin was wiped with water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made immediately (1 – 2 hours) after treatment, and thereafter at least once a day. The animals were sacrificed at the end of the 14–day observation period and subjected to a gross necropsy.

II. RESULTS AND DISUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14–day observation period.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

Severe lung congestion, splenomegaly, hepatomegaly with centrilobular congestion, and subcapsular renal petechiae were observed in all male and female animals.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except to minor deviations, the study is in accordance to the OECD 402 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Due to the limitations (minimal reporting and occlusive dressing) the study is considered acceptable (reliable with restrictions). The reported purity is low, however, this is due to the use of the IPA salt form of glyphosate. Under the study conditions the acute dermal LD_{50} > 2000 mg/kg bw in males and female rats.

B.6.2.2.15. Study 15

Data point	CA 5.2.2/015
Report author	[redacted]
Report year	1994
Report title	Acute dermal toxicity of glyphosate technical in the rat
Report No	T1586.3.A
Document No	2332616
Guidelines followed in study	Not reported
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Limited reporting. Approximately 10% of the total body surface should be exposed, which is about 32-42 cm² for rats. In this study only an area of 8 cm² was exposed.
Previous evaluation	Yes, accepted in RAR (2015)
GLP	No, Quality assurance statement available.
Acceptability/Reliability	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2002, *Category 4b* **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written below was prepared by the AGG. The study is considered to be unacceptable, since the study was not performed according to GLP and due to the limited reporting.

Short description of study design and observations	Tested material: Glyphosate technical Purity of test compound: 95 % Vehicle: suspended (50 % w/w) in natrosol (1 % w/w in water) Test animals: 5 rats (Sprague-Dawley)/sex, weighing between 200 and 230 grams at the beginning of the experiment. The source of the animals was [redacted]. The rats were fed on a diet and tap water *ad libitum*. They were acclimatised to the laboratory for 5 days. The animals were shaven and the test sample was suspended at 50% w/w in natrosol [1% w/w in tap water] and applied dermally at 4 g/kg. Approximately an area of 8 cm² was exposed. Dose level: 2000 mg/kg bw. The test substance was rubbed into the skin with a metal spatula for 120 seconds, left to dry for 60 seconds and then covered with a gauze patch (Nu Gauze), which was attached with hypoallergenic tape (Dermicel). The gauze-dermicel patch was held in contact with the skin by means of hypoallergenic tape (Micropore, 3M) as a semi-occlusive dressing. At the end of the exposure period (24 hours), the dressing was removed and residual test substance was removed with water. Animal observations were made soon after dosing; than at frequent intervals during the first day and daily after the first day. Clinical signs were recorded individually at each observation. Animal weights were recorded before administration, after 7 days and on completion of the experiment at day 15. All animals were submitted to gross necropsy

	and individual pathology records were made for the liver, kidneys, adrenals, gonads of all animals surviving 24 hours or more.
Short description of results	No mortality, abnormal clinical signs, weight loss and no abnormalities were found in the autopsy of the major organs LD_{50} >2000 mg/kg bw (limit test)
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL

Assessment and conclusion by RMS:
In contrary to the previous evaluation (RAR, 2015) the study is considered unacceptable. The study is not performed according to GLP and the exposed area is too small to draw conclusions.

B.6.2.2.16. Study 16

Data point	CA 5.2.2/016
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute dermal toxicity (limit test) in the rat
Report No	710/15
Document No	2332786
Guidelines followed in study	OECD 402 (1987), Method B3 (Commission Directive 92/69/EEC)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. The study reporting is very minimal. Individual animal data were not provided. The skin of the rats is moistened with arachis oil B.P prior to treatment. Solid test substances should be applied using a vehicle and not by moistening the skin pre-treatment, because this could create a barrier that prohibits contact of the test substance with the skin. Since, this deviation could impact the outcome of the study, it is considered unacceptable.
Previous evaluation	Yes, accepted in RAR (2015)
GLP	Yes
Acceptability/Reliability	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2002, *Category 4b* **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered unacceptable due to way the test substance is applied and the limited reporting.

Short description of study design and observations	Test material: Glyphosate 62% IPA, batch 9409/50 Purity of test compound: 95 % Vehicle: none Test animals: 5 rats (Sprague-Dawley)/sex Dose level: 2000 mg/kg bw A group of ten animals (five males and five females) was given a single 24-hour, semi-occluded dermal application to intact skin at a dose level of 2000 mg/kg bw. The animals were observed for fourteen days after the day of treatment and were then killed for gross pathological examination
Short description of results	There were no deaths, no signs of systemic toxicity or skin irritation. All animals showed expected gain in bodyweight during the study. No abnormalities were noted a necropsy. LD_{50} >2000 mg/kg bw (limit test)
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL

Assessment and conclusion by RMS:
In contrary to the previous evaluation (RAR, 2015) the study is considered unacceptable. The test substance is applied after previous moistening of the skin with oil which could create a barrier between the skin and the test substance. This in combination with the limited reporting is why no conclusion could be drawn.

B.6.2.2.17. Study 17

Data point	CA 5.2.2/017
Report author	[redacted]
Report year	1994
Report title	Glyphosate ([redacted]): Acute Dermal Toxicity in Rats
Report No	[redacted]-94-402/R
Document No	Not reported
Guidelines followed in study	OECD 402 (1981)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Additional five rats per sex served as a control group. Age of animals was not reported. Body weight of female animals was outside of the range specified in the guideline (200 – 300 g). No observations during the first 30 minutes after dosing were made, but only once between one four hours after dosing. Size of exposed skin area and the amount of vehicle used was not reported. Instead of a semi-occlusive dressing, aluminium foil was used which is considered occlusive.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, Quality assurance statement available.
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered unacceptable, since it was not performed according to GLP.

The acute dermal toxicity potential of the test material, glyphosate technical (Batch: 36300892; Purity: 99.6 %), was investigated in five male and five female LATI/Wistar rats per dose group. The test substance was applied at

doses of 0 and 2000 mg/kg bw (limit test) to clipped dorsal skin and covered by occlusive dressing for 24-hours. After exposure the test substance was removed with water and the rats were observed for 14 days.

Mortality and clinical signs of toxicity were monitored on the day of administration and daily thereafter. All animals were subjected to gross necropsy.

No mortality and clinical symptoms occurred during the study. No differences were noted in body weight. The gross necropsy demonstrated no gross changes attributable to the test material. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	99.6 %
Stability of test compound:	Expiry date: 1994-09-01

2. Vehicle and/or positive control: Water

3. Test animals:

Species:	Rat
Strain:	LATI/Wistar
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	Male: 210 – 215 g; female: 150 – 155 g
Acclimation period:	22 days
Diet/Food:	Altromin rodent chow, *ad libitum*
Water:	Daily changed tap water in bottles, *ad libitum*
Housing:	Macrolon III. box
Environmental conditions:	Temperature: 20 ± 2 °C
	Humidity: 45 – 70 %
	Air changes: 10 / hour
	Photocycle: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1994-01-19 to 1994-02-02

Animal assignment and treatment:

Before the application of the test material, the fur was removed by clipping the dorsal skin of five male and five female rats.

The test substance was administered dermally in a single application at a dose level of 0 and 2000 mg/kg bw (limit test) at a concentration of 33.3 % in an aqueous solution. The test substance was held in contact with the skin by means of non-absorbent binder of polyethylene.

After 24-hours, the binders were removed, and the treated skin was wiped with water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made immediately after dosing, at 1 and 4 hours postdose, and once daily thereafter for 14 consecutive days. The animals were sacrificed at the end of the 14-day observation period. Gross changes in the thoracic and abdominal organs as well as selected organ weights were recorded.

II. RESULTS AND DISUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14–day observation period.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No gross changes attributable to the test material were observed in treated and control animals. The absolute and relative organ weights revealed no differences attributable to the test material.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except to minor deviations, the study is in accordance to the OECD 402 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. The acute dermal LD_{50} is greater than 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
In contrary to the previous evaluation (RAR, 2015) the study is considered unacceptable. The study is not performed according to GLP and thus no conclusion can be drawn.

B.6.2.2.18. Study 18

Data point	CA 5.2.2/018
Report author	[redacted]
Report year	1992
Report title	Glyphosate technical: Acute dermal toxicity (limit test) in the rat
Report No	134/38
Document No	Not reported
Guidelines followed in study	OECD 402 (1981)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Animals were 10-14 weeks old instead of 8-10 weeks. Purity and stability of test substance was 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 to be acceptable (reliable with restrictions), since the purity and stability of the test substance were not reported.

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated in five male and five female Sprague-Dawley rats per dose. The test substance was applied to clipped dorsal skin at doses of 2000 mg/kg bw. After 24-hours exposure duration the test substance was removed and the rats were observed for 14 days. No mortality, clinical symptoms and local skin reactions occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. All animals showed expected gain in body weight. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate technical
Description: White powder
Lot/Batch #: L3258
Purity: Not specified
Stability of test compound: Not specified

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species: Rat
Strain: Sprague-Dawley
Source: [redacted]

Age:	10 – 14 weeks	
Sex:	Males and females	
Weight at dosing:	Males: 219 – 247 g; females: 200 – 215 g	
Acclimation period:	At least 5 days	
Diet/Food:	Rat and Mouse Expanded Diet No. 1 (Special Diet Services Limited, Witham, Essex, UK, *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Solid-floor polypropylene cages with softwood sawdust bedding; individually housed during the exposure. For the remainder of the study, housed five animals per sex per cage in polypropylene grid-floor cages suspended over trays lined with absorbent paper.	
Environmental conditions:	Temperature:	19 – 22 °C
	Humidity:	52 – 63 %
	Air changes:	15 per hour
	Photoperiod:	12 hour light / dark cycle

B: Study design and methods

In life dates: 1992-01-06 to 1992-01-20

Animal assignment and treatment:

On the day before application of the test material, a group of five male and five female rats per dose was prepared by clipping the backs and flanks free of hair (approximately 10 % of the total body surface area).

Glyphosate technical was administered dermally in a single application under semi-occlusion at a dose level of 2000 mg/kg bw. On Day 0 the undiluted test material was applied onto the shorn skin of rats, previously moisted with distilled water, surgical gauze was placed over the treatment area, and the area was semi-occluded with a piece of self-adhesive bandage (HYPERTIE). The bandage was further secured with a piece of BLENDERM wrapped around each end.

After 24-hours the bandage was removed and the treated skin was wiped with cotton wool moistened with distilled water to remove any residual test material.

Observations for mortality and clinical signs of toxicity were made 0.5, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. The rats were weighed immediately prior to dosing, 7 and 14 days after dosing. The animals were sacrificed at the end of the 14-day observation period and subjected to a gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed during the 14 days observation period. No treatment related skin irritation was observed in any animal throughout the study.

C. BODY WEIGHT

All animals gained weight over the study period (see table below).

Table 6.2.2.18-7 Glyphosate technical: Acute Dermal Toxicity (Limit test) in the Rat [redacted] 1992): Mean body weight

Dose level	2000 mg/kg bw									
Sex	Males			Females			Males		Females	
Day	0	7	14	0	7	14	0 – 7	0 – 14	0 – 7	0 – 14
	Body weight [g]						Body weight gain [g]			
Mean	233.8	283.8	334.0	208.0	225.8	246.8	50.0	100.2	17.8	38.8
± SD	± 11.3	± 19.6	± 27.5	± 6.6	± 8.3	± 9.6	± 15.0	± 21.0	± 5.9	± 7.7

Note: Body weight and body weight gain values were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormalities were observed during necropsy.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance to the OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
In contrary to the previous evaluation (RAR, 2015) the study is considered acceptable (reliable with restrictions) due to the missing information on purity and stability of the test substance and considering that there are studies available without these limitations.

B.6.2.2.19. Study 19

Data point	CA 5.2.2/019
Report author	[redacted]
Report year	1991
Report title	Acute dermal toxicity study with glyphosate technical (FSG 03090 H/05 March 90) in Wistar rats
Report No	[redacted]876.ADR
Document No	Not reported
Guidelines followed in study	OECD 402 (1987)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Animals were 10-14 weeks old instead of 8-10 weeks. Two high doses (above limit dose of 2000 mg/kg bw) were selected (2500 and 5000 mg/kg bw). Weight of male (150 - 200 g) and female animals (150 - 180 g) was outside of the range specified in the guideline (200 - 300 g). Time of observation on the day of application was not specified according to the test guideline. Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no mortality or toxicity was reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations 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 to be acceptable.

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated in five male and five female Wistar rats per dose. The test substance was applied to clipped dorsal skin at doses of 2500 and 5000 mg/kg bw. After 24-hours exposure duration the test substance was removed and the rats were observed for 14 days. No mortality, clinical symptoms and local skin reactions occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Some of the animals showed body weight losses. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	60
Purity:	96.8 %
Stability of test compound:	More than two years (declared), expiry date: July 1992

2. **Vehicle and/ or positive control:** Distilled water
3. **Test animals:**

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	14 weeks
Sex:	Males and females
Weight at dosing:	Males: 150 – 200 g; females: 150 – 1 80 g
Acclimation period:	At least 1 week
Diet/Food:	Standard 'Gold Mohur' brand pelleted rat feed (M/s Lipton India Ltd., Bangalore, India), *ad libitum*
Water:	Deep bore well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individual polypropylene cages with steam sterilized clean paddy husk bedding
Environmental conditions:	Temperature: 23 ± 2 °C
	Humidity: 68 ± 6 %
	Air changes: 10 – 15 / hour
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: October 1990 (no details given)

Animal assignment and treatment:

Approximately 24-hours before application of the test material, a group of five male and five female rats per dose was prepared by clipping the backs free of hair (approximately 10 % of the total body surface area).

Glyphosate Technical was administered dermally in a single application under occlusion at a dose level of 2500 and 5000 mg/kg bw. On test day one (Day 0) the test material was made into a slurry with distilled water on aluminium foil. The test material preparation along with foil was applied to the prepared area of the skin of rats and fixed with tape (USP; Johnsonplast). After 24-hours the tape was removed and the skin was rinsed with luke warm water, washed with 1 % Labklin, and rinsed with luke warm water. Skin was wiped dry with a cotton hand towel.

Observations for mortality and clinical signs of toxicity were made four times during Day 0 and daily during Days 1 – 14. The rats were weighed immediately prior to dosing, 7 and 14 days after dosing. The animals were sacrificed at the end of the 14-day observation period and subjected to a gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Single dermal administrations of 2500 or 5000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity. No skin reactions were observed.

C. BODY WEIGHT

Body weight gains, with the exception of some animals which lost weight, were acceptable. One rat in the 2500 mg/kg bw dose group and five rats in 5000 mg/kg bw dose group had lost body weight at Day 7. At Day 14 one rat each in 2500 and 5000 mg/kg bw dose group had lost body weight, while the remaining rats had gained body weight. A summary of the body weights is given in the table below.

Table 6.2.2.19-8 Acute Dermal Toxicity Study with glyphosate technical (FSG 03090 H/05 March 90) in Wistar rats ([redacted] 1991): Body weight and body weight gain

Dose level	**2500 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
41 M (46 F)	190	192	216	184	188	180	26	-4
42 M (47 F)	150	150	166	168	172	184	16	16
43 M (48 F)	180	184	194	182	184	200	14	18
44 M (49 F)	200	222	252	170	174	174	52	4
45 M (50 F)	170	160	176	166	170	188	6	22
Mean	**178.0**	**181.6**	**200.8**	**174.0**	**177.6**	**185.2**	**22.8**	**11.2**
± SD	**± 19.2**	**± 28.3**	**± 34.4**	**± 8.4**	**± 7.9**	**± 9.8**	**± 17.8**	**± 10.8**
Dose level	**5000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
51 M (56 F)	154	140	170	156	160	164	16	8
52 M (57 F)	178	184	210	154	164	170	32	16
53 M (58 F)	174	154	180	170	172	180	6	10
54 M (59 F)	182	178	192	176	172	180	10	4
55 M (60 F)	174	174	190	170	160	168	16	-2
Mean	**172.4**	**166.0**	**188.4**	**165.2**	**165.6**	**172.4**	**16.0**	**7.2**
± SD	**± 10.8**	**± 18.4**	**± 14.9**	**± 9.7**	**± 6.1**	**± 7.3**	**± 9.9**	**± 6.7**

§ = animal No. of males and (females). All animal numbers are preceeded by "A 03", and have been truncated to the last two digits in this table (i.e. animal A 0341 is listed as 41 in this table).
Note: Mean and standard deviation as well as body weight gain data were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female Wistar rats, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 5000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.20. Study 20

Data point	CA 5.2.2/020
Report author	[redacted]
Report year	1990
Report title	Acute dermal toxicity study in the rat: Glyphosate technical.
Report No	[redacted] 900823A
Document No	Not reported
Guidelines followed in study	OECD 402 (1981), EC method B3 (84/449/EEC, 1984)
Deviations from current test guideline (OECD 402, 2019)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Three high doses (above limit dose) were selected. Age of animals was not reported. Body weight is not within the interval of ± 20 % of the mean weight. Temperature was in the range of 13 – 25 °C instead of 22 ± 3 °C. The number of air changes was not specified. No justification for use of vehicle and justification for choice of vehicle was given. Individual animal data were not provided for some endpoints. Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no mortality or toxicity was reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	The study was conducted in compliance with GLP with the exceptions that the environmental conditions were outside the specified range and some aspects of study documentation were not in accord with GLP
Acceptability/Reliability	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated following single application to groups of 10 rats (five males, five females). The test substance was applied to shaved skin at doses of 3000, 5000 and 8000 mg/kg bw. A dry gauze pad was used as the control substance. After 24-hours contact period the test substance was removed and the rats were observed for 14 days. There were no mortality, clinical signs of toxicity, or necropsy findings. Changes of body weight were in the normal range. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 8000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Not specified
Lot/Batch #:	0190 A
Purity:	98.1 %
Stability of test compound:	Stable at room temperature (declared)

2. **Vehicle and/ or positive control:** 0.9 % saline
3. **Test animals:**

Species:	Rat
Strain:	CD
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Male: 220 – 301 g; female: 200 – 225 g
Acclimation period:	5 days
Diet/Food:	Standard rat diet pellets (Redmills, Goresbridge, Co. Kilkenny, Ireland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual polypropylene cages with stainless steel lids and autorclaved shaving bedding
Environmental conditions:	Temperature: 13 – 25 °C
	Humidity: 43 – 63 %
	Air changes: Not specified
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1990-03-15 to 1990-08-23

Animal assignment and treatment:

Approximately 24-hours before application of the test material, a group of five male and five female rats per dose group was prepared by shaving the backs free of hair. Only animals with healthy intact skin were used.

Glyphosate technical was administered by direct application to the skin at dose levels of 3000, 5000 and 8000 mg/kg bw. Each animal received a single administration of glyphosate technical. The test material was moistened using 0.9 % saline and applied to the gauze pad. The gauze pad was held in place by an adhesive tape covered with self-sealing plastic. A final covering was made with adhesive tape which was wrapped completely around the rat. After 24-hours the dressing was removed and residual test substance was washed off with water. A dry gauze pad was applied to the control group which was otherwise treated identically to the test group.

Observations for mortality and clinical signs of toxicity were made 20 – 30 minutes, 60 – 70 minutes and 3 – 3.5 hours post dosing and daily thereafter. For each animal body weight was recorded before dosing (Day 0), on Day 7 and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Single dermal administrations of 3000, 5000 and 8000 mg/kg bw to five male and five female rats did not reveal any clinical signs of toxicity.

C. BODY WEIGHT

All animals gained their body weights on Days 7 and 14 after administration. Thus, body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female CD rats, observed over a period of 14 days was greater than 8000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 8000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 8000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.21. Study 21

Data point	CA 5.2.2/021
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: Acute dermal toxicity (limit) test in rats
Report No	5884
Document No	Not reported
Guidelines followed in study	OECD, EEC, EPA[1]
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rats of both sexes instead of two animals, preferred females, were used per dose. Purity of test substance as well as amount of vehicle used (i.e. water) were not reported. From the batch number a purity of 98.6 % was concluded. Weight of female animals was outside of the range specified in the guideline (200 – 300 g). Number of air changes was not specified. Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no mortality was reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations 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 to be acceptable.

[1] Guideline numbers are not specified in the report, however the study is compliant with OECD 402, EEC B3, and EPA 81-2 with the exception of a slightly different test item application procedure.

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated in five male and five female Sprague-Dawley rats. The test substance was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw. Clinical signs noted at 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at the test sites 2 – 14 days after dosing. No mortality occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Body weight gains with the exception of one animal, which lost weight, were acceptable. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical (PMG)
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	Not specified in the study report Batch 206-JaK-25-1 reported with 98.6 %, see [redacted] 1991(see CA 5.2.1/25).
Stability of test compound:	No data given in the report

2. Vehicle and/ or positive control: Water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	8 - 10 weeks
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 212 - 240 g; females: 188 - 234 g
Acclimation period:	6 days
Diet/Food:	Expanded Rat and Mouse Maintenance Diet (Special Diets Services), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Polypropylene cages with mesh floors in groups of five animals per sex per cage; individually housed during exposure (24-hours)
Environmental conditions:	Temperature: 19 °C and 22 °C (min. and max. average)
	Humidity: 49 % (average)
	Air changes: Not specified
	Photoperiod: 12-hour light/dark cycle (light 07:00 – 19:00)

B: Study design and methods

In life dates: 1989-06-06 to 1989-06-21 (Start of treatment to study completion)

Animal assignment and treatment:

A group of five male and five female rats was prepared by clipping the backs free of hair, approximately 24-hours before application of the test material. Care was taken to avoid abrading the skin. Glyphosate technical was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw.

The test material, moistened with water, was applied evenly onto gauze dressing, which was applied to the shaved back of each rat. At least 10 % of the body surface was in contact with the test material. The trunk of the rat was then encircled with a strip of non-irritating tape (Sleek). After 24-hours the tape was removed, and the skin was wiped with a water-dampened tissue to remove excess test material.

The rats were observed frequently on the day of dosing and once daily for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14-day observation period.

At the end of the observation period and sacrifice by carbon dioxide asphyxiation, each animal was subjected to a gross post mortem examination.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs noted 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at test sites 2 to 14 days after dosing (see table below).

Table 6.2.2.21-9 Glyphosate technical: Acute Dermal Toxicity (limit) test in Rats [redacted] 1989): Clinical observation data

Sex	Observation	Animals affected / Time (after dosing)								
		1 m	30 m	1 h	2 h	1 d	2 d	3 d	4 d	5 - 14 d
Males	NAD	5/5					5/5	5/5	5/5	5/5
	Pilorection		5/5	5/5	5/5	5/5				
	Reduced activity		5/5	5/5	5/5					
	Scab formation						1/5*	1/5*	1/5*	1/5*
Females	NAD	5/5					5/5	5/5	5/5	5/5
	Pilorection		5/5	5/5	5/5	5/5				
	Reduced activity		5/5	5/5	5/5					
	Scab formation						2/5*	2/5*	2/5*	2/5*

NAD = No abnormalities detected; m = minute; h = hour; d = day
* = Animals NAD but scab formation noted at test sites

C. BODY WEIGHT

Body weight gains with the exception of one animal, which lost weight, were acceptable. A summary of the body weights is given in the table below.

Table 6.2.2.21-10 Glyphosate technical: Acute Dermal Toxicity (limit) test in Rats [redacted] 1989): Body weight and body weight gain

Dose level	2000 mg/kg bw							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 - 14**	**0 - 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
11 M (16 F)	232	268	300	234	259	229	68	-5
12 M (17 F)	240	289	336	188	213	278	96	90
13 M (18 F)	212	252	283	204	216	243	71	39
14 M (19 F)	237	285	313	207	226	248	76	41
15 M (20 F)	224	271	320	208	227	241	96	33
Mean	**229**	**273**	**310**	**208**	**228**	**248**	**81**	**40**
± SD	**± 11**	**± 15**	**± 20**	**± 17**	**± 18**	**± 18**	**± 14**	**± 34**

§ = animal No. of males and (females)

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female SD rats, observed over a period of 14 days was greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 2000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the dermal LD_{50} > 2000 mg/kg bw in males and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.2.22. Study 22

Data point	CA 5.2.2/022
Report author	[redacted]
Report year	1989
Report title	Acute dermal toxicity study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of Nphosphonomethylglycine acid) in rats
Report No	238061
Document No	PRO425
Guidelines followed in study	No final conclusion possible.
Deviations from current test guideline (OECD 402, 2017)	No assessment possible
Previous evaluation	Yes, accepted in RAR (2015)
GLP	Yes
Acceptability/Reliability	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b* **Conclusion AGG:** Study not acceptable as study report could not be retrieved.

Short description of study design and observations	Purity of test compound: 62 % (IPA) Vehicle: none Test animals: 5 rats (Wistar)/sex Dose level: 2000 mg/kg bw
Short description of results	Erythema maculate (1 ♂), scales (1 ♀) LD_{50} > 2000 mg/kg bw (limit test)
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS: The study report could not be retrieved by the RMS. Therefore, no conclusion could be drawn.

B.6.2.2.23. Study 23

Data point	CA 5.2.2/023
Report author	[redacted]
Report year	1988
Report title	Acute dermal toxicity study of glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits
Report No	88.2053.008
Document No	[redacted] 88-29
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals [Acute Dermal Toxicity, 81-2] (1984)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. Age of animals was not reported. Number of air changes was not specified. No justification for use of vehicle and justification for choice of vehicle was given. Observation time on the day of application not specified as recommended in the test guideline. Even though there was just one obvious abnormality, individual animal data showing necropsy findings were not reported. Instead of a semi-occlusive dressing an occlusive dressing was used, which is considered worst-case.
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 (reliable with restrictions) due to the limited reporting and the occlusive dressing used.

The acute dermal toxicity potential of the test material glyphosate was investigated following single application to 10 rabbits (five males, five females). The test substance was applied to clipped skin at a dose of 5000 mg/kg bw. After 24-hours contact period, the test substance was removed and the rabbits were observed for 14 days. One female rabbit was found dead on Day 13. The female that died exhibited diarrhea and anorexia. No internal abnormalities were noted during gross examination. Therefore, this death was not considered treatment related. Further, anorexia (one male, one female), diarrhea (one male, one female) and soft stool (one female) were noted in the animals which survived to study termination. Test substance application caused no adverse effect on mean body weight in either sex. One male had a white caseous substance adhered to the lungs, which was not considered treatment-related. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate

Description: White powder

Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	Physiological saline
3. Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.36 – 2.49 kg; females: 2.34 – 2.97 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Fresh tap water, *ad libitum*
Housing:	Individual wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C
	Humidity: 40 – 60 %
	Air changes: Not specified
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1988-04-11 to 1988-04-25

Animal assignment and treatment:

Approximately 24-hours before application of the test material, five male and five female rabbits were prepared by clipping the backs (from the shoulders to the hindquarters, approximately 10 % of the total body surface) free of hair. Only animals with healthy intact skin were used.

Glyphosate was moistened with physiological saline (approximately 1 mL/g of test substance) and applied topically to the clipped area at a dose level of 5000 mg/kg bw. Each test site was covered with gauze and occluded with a layer of plastic wrap and stockinette sleeve held in place with tape. After 24-hours the binders were removed and the exposure sites were gently wiped with gauze.

Observations for mortality and clinical signs of toxicity were made three times on the day of administration and twice daily thereafter. For each animal body weight was recorded before dosing (Day 0), on Day 7 and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

One female rabbit was found dead on Day 13 following test substance administration. All other rabbits survived to study termination on Day 14.

B. CLINICAL OBSERVATIONS

The female rabbit that died exhibited diarrhea and/or anorexia on Days 8 – 12. These observations are consistent with mucoid enteropathy, a condition occasionally noted in stock laboratory rabbits. Anorexia (one male, one female), diarrhea (one male, one female) and soft stool (one female) were noted in the animals that survived to study termination. Clinical observations are summarised in the table below.

Table 6.2.2.23-11: **Acute Dermal Toxicity Study of Glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits [redacted] 1988): Clinical observation data**

Dose level (mg/kg bw)	**5000**	
Sex	**Males**	**Females**
Clinical observation	**Incidence***	
Anorexia	1/5[a] (Day 1 – 8)	1/5 (Day 8 – 12)
Diarrhea	1/5 (Day 5 – 8)	1/5 (Day 9 – 12)
Soft stools	-	1/5 (Day 11, 12) 1/4 (Day 13)

* = number of observed clinical signs/number of surviving animals, point in time (day) in parenthesis
- = not observed

C. BODY WEIGHT

All surviving animals maintained or gained weight over the study period with the exception of one male rabbit, which had slight weight loss. A summary of the body weights is given in the table below.

Table 6.2.2.2-12 Acute Dermal Toxicity Study of Glyphosate batch/lot/NBR No. XLI-55 in New Zealand White rabbits [redacted] 1988): Body weight and body weight gain

Dose level	**5000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 – 14**	**0 – 14**
Animal No.[§]	**Body weight [g]**						**Body weight gain [g]**	
1169 (1245)	2.49	2.69	2.40	2.34	2.49	2.64	-0.09	0.30
1173 (1247)	2.46	2.60	2.75	2.60	2.71	2.77	0.29	0.17
1174 (1250)	2.44	2.51	2.69	2.74	3.05	3.21	0.25	0.47
1175 (1251)	2.38	2.36	2.61	2.69	2.73	2.89	0.23	0.20
1184 (1254)	2.36	2.07	2.43	2.97	2.82	-[a]	0.07	-
Mean	**2.43**	**2.45**	**2.58**	**2.67**	**2.76**	**2.88**	**0.15**	**0.29**
± SD	**± 0.05**	**± 0.24**	**± 0.16**	**± 0.23**	**± 0.20**	**± 0.24**	**± 0.16**	**± 0.14**

§ = animal No. of males and (females)
a = Animal found dead on Day 13 (final body weight 2.45 kg).
Note: Body weight gain data were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormalities were noted during gross necropsy examination of the rabbit that died. One male rabbit that was sacrificed at study termination had a white caseous substance adhered to the lungs. In the study report this finding is not discussed. Nevertheless, rabbits have been observed to develop a disease showing caseous necrosis after bacterial infections. The finding is not considered treatment-related.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after a single dermal administration to male and female New Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Contrary to the conclusion by the applicant and the conclusion in the RAR (2015) the study is considered acceptable with restrictions due to the limited reporting and the occlusive dressing used. Although one female animal died during the study period and the study was performed with a worst-case occlusive dressing it is agreed with the conclusion by the applicant. The the acute dermal LD_{50} in male and female rabbits is > 5000 mg/kg bw.

B.6.2.2.24. Study 24

Data point	CA 5.2.2/024
Report author	[redacted]
Report year	1987
Report title	Acute dermal toxicity study of MON 8722 in New Zealand White rabbits
Report No	9307A
Document No	[redacted] 86-430
Guidelines followed in study	US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals [Acute Dermal Toxicity, 81-2] (1982)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. Age of animals was not reported. Number of air changes was not specified. Justification for use of vehicle and justification for choice of vehicle missing. Observation time on the day of application not specified as recommended in the test guideline. Even though there were only a few sporadic clinical observations and no obvious abnormalities, individual animal data showing clinical signs of toxicity findings and time course of onset were not reported. Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and no mortality was reported, this deviation is not considered to impact the outcome of the study, similarly to the other deviations 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 to be acceptable (reliable with restrictions) due to the limited reporting and the occlusive dressing used.

The acute dermal toxicity potential of the test material MON 8722 was investigated following single application to 10 rabbits (five males and five females). The test substance was applied to clipped skin at a dose of 5000 mg/kg bw. After 24-hours contact period the test substance was removed and the rabbits were observed for 14 days. No mortality occurred. Soft stools were transiently noted in one animal per sex. Test substance administration did not produce an adverse effect in weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: MON 8722

Description:	White powder
Lot/Batch #:	XLG-256
Purity:	70.7 %
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: Physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.31 – 2.64 kg; females: 2.18 – 2.67 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Fresh tap water, *ad libitum*
Housing:	Individual wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 °C
	Humidity: 40 – 60 %
	Air changes: Not specified
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1986-11-07 to 1986-11-21

Animal assignment and treatment:

Approximately 24-hours before application of the test material, five male and five female rabbits were prepared by clipping the backs (from the shoulders to the hindquarters, approximately 10 % of the total body surface) free of hair. Only animals with healthy intact skin were used.

MON 8750 was mixed with physiological saline (1 mL/g) and applied topically to the clipped area at a dose level of 5000 mg/kg bw. Each test site was wrapped with occlusive binders consisting of a layer of plastic wrap and stockinette sleeve held in place with the tape. After 24-hours the binders were removed and the exposure sites were gently wiped with gauze.

Observations for mortality and clinical signs of toxicity were made frequently on the day of administration and twice daily thereafter. For each animal body weight was recorded before dosing (Day 0), on Day 7 and on Day 14. The animals were sacrificed at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred. All rabbits survived to study termination on Day 14.

B. CLINICAL OBSERVATIONS

Soft stools were transiently noted in one animal per sex (duration and timing not reported). All animals appeared normal at study termination.

C. BODY WEIGHT

Body weight development was not affected by the treatment in either sex.

D. NECROPSY

No macroscopic pathologic abnormalities were noted during gross necropsy examination in any animal.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female New Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
In contrary to the previous evaluation (RAR, 2015) the study is considered acceptable (reliable with restrictions) due to the limited reporting and the worst-case occlusive dressing used in the study. The study results confirm the LD_{50} > 5000 mg/kg bw.

B.6.2.2.25. Study 25

Data point	CA 5.2.2/025
Report author	[redacted]
Report year	1987
Report title	Acute dermal toxicity study of MON-8750 in New Zealand White rabbits
Report No	9308A
Document No	[redacted] 86-431
Guidelines followed in study	US EPA OPP 81-2, Acute Dermal Toxicity (1982)
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. Age of animals was not reported. Number of air changes was not specified. Justification for use of vehicle and justification for choice of vehicle is missing. Observation time on the day of application not specified as recommended in the test guideline. Even though there were only a few sporadic clinical observations and no obvious abnormalities, individual animal data showing clinical signs of toxicity findings and time course of onset were not reported. Instead of a semi-occlusive dressing an occlusive dressing was used, which is considered worst-case.
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 to be acceptable (reliable with restrictions) due to the limited reporting and the occlusive dressing.

The acute dermal toxicity potential of the test material MON 8750 was investigated following single application to 10 rabbits (five males, five females). The test substance was applied to clipped skin at a dose of 5000 mg/kg bw. After 24-hours contact period, the test substance was removed and the rabbits were observed for 14 days. One female rabbit was found dead on Day 3, however, this death was not considered treatment related. The female that died exhibited anorexia and decreased activity. Further, diarrhea and soft stools were noted in three male rabbits and three female rabbits. All surviving animals maintained or gained weight over the study period, with the exception of two males that had slight weight loss. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: MON 8750
Description: White powder
Lot/Batch #: XLG-255
Purity: 90.8 %

Stability of test compound:	Not specified
2. Vehicle and/ or positive control:	Physiological saline
3. Test animals:	
Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.26 - 2.57 kg; females: 2.20 - 2.59 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Fresh tap water, *ad libitum*
Housing:	Individual wire mesh cages
Environmental conditions:	Temperature: 20 - 23.9 °C
	Humidity: 40 - 60 %
	Air changes: Not specified
	Photoperiod: 12-hour light/dark cycle

B: Study design and methods

In life dates: 1986-11-07 to 1986-11-21

Animal assignment and treatment:

Approximately 24-hours before application of the test material, five male and five female rabbits were prepared by clipping the backs (from the shoulders to the hindquarters, approximately 10 % of the total body surface) free of hair. Only animals with healthy intact skin were used.

MON 8750 was mixed with physiological saline (1 mL/g) and applied topically to the clipped area at a dose level of 5000 mg/kg bw. Each test site was wrapped with occlusive binders consisting of a layer of plastic wrap and stockinette sleeve held in place with tape. After 24-hours the binders were removed and the exposure sites were gently wiped with gauze.

Observations for mortality and clinical signs of toxicity were made frequently on the day of administration and twice daily thereafter. For each animal bodyweight was recorded before dosing (Day 0), on Day 7, and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

One female rabbit was found dead on Day 3. All other rabbits survived to study termination on Day 14.

B. CLINICAL OBSERVATIONS

The female rabbit that died exhibited anorexia and decreased activity prior to death. Diarrhea and soft stools were sporadically noted in three males and three females during the observation period. The timing and duration of these effects were not reported nor was the individual data available.

C. BODY WEIGHT

All surviving animals maintained or gained weight over the study period with the exception of two male rabbits, which had slight weight loss. A summary of the body weights is given in Table 6.2.2.25-13.

Table 6.2.2.25-13 Acute Dermal Toxicity Study of MON-8750 in New Zealand White rabbits [redacted] 1987): Body weight and body weight gain

Dose level	**5000 mg/kg bw**							
Sex	**Males**			**Females**			**Males**	**Females**
Day	**0**	**7**	**14**	**0**	**7**	**14**	**0 - 14**	**0 - 14**
Animal No.§	**Body weight [g]**						**Body weight gain [g]**	
3325 (3400)	2.26	2.42	2.56	2.37	2.54	2.67	0.30	0.30
3326 (3401)	2.36	2.47	2.49	2.59	2.74	2.87	0.13	0.28
3327 (3402)	2.29	2.39	2.30	2.20	2.43	2.61	0.01	0.41
3328 (3403)	2.37	2.55	2.70	2.38	-[a]	-	0.33	-
3329 (3404)	2.57	2.58	2.56	2.48	2.53	2.76	-0.01	0.28
Mean	**2.37**	**2.48**	**2.52**	**2.40**	**2.56**	**2.73**	**0.15**	**0.32**
± SD	**± 0.12**	**± 0.08**	**± 0.15**	**± 0.14**	**± 0.13**	**± 0.11**	**± 0.16**	**± 0.06**

§ = animal No. of males and (females)
a = Animal found dead on Day 3
Note: Body weight gain data were not given in the study report. Values were calculated retrospectively.

D. NECROPSY

No abnormalities were noted during gross necropsy examination in any animal.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female NEW Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD 402 (2017). Therefore, the outcome can be reported as valid. The acute dermal LD_{50} is above 5000 mg/kg bw. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:

In contrary to the previous evaluation (RAR, 2015) the study is considered acceptable (reliable with restrictions) due to the low purity, the limited reporting and the worst-case occlusive dressing used in the study. The study results confirm the LD_{50} > 5000 mg/kg bw.

B.6.2.2.26. Study 26

Data point	CA 5.2.2/026
Report author	[redacted]
Report year	1983
Report title	The acute dermal toxicity (LD_{50}) to rabbits with glyphosate (tech) of [redacted]
Report No	Not reported
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 402, 2019)	Two rabbits of both sexes were used. The following information were not given: age of animals, housing conditions, size of exposed skin area (i.e. at least 10 % of total body surface area), type and amount of vehicle, acclimation period and stability of the test compound. Body weights of animals after administration of the test substance were not determined. Animals were not subjected to gross necropsy. Individual animal data showing signs of toxicity were not reported. Additionally, a solvent control group was included which is not a requirement.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facility (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is unacceptable

The acute dermal toxicity potential of the test material, glyphosate technical, was investigated following single application to four rabbits (two males, two females). The test substance was applied to shaved skin at a dose of 2000 mg/kg bw. After 24-hours contact period the test substance was removed and the rabbits were observed for 14 days. No mortality occurred. Slight erythema at the test site of treatment was observed for 24-hours. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical
Description:	White amorphous powder
Lot/Batch #:	R & D sample (8.7.83.)
Purity:	95 %
Stability of test compound:	Not specified

2. **Vehicle and/ or positive control:** No information provided
3. **Test animals:**

Species:	Rabbit

Strain:	Albino NWS	
Source:	[redacted]	
Age:	Not specified	
Sex:	Males and females	
Weight at dosing:	1.5 – 2.5 kg	
Acclimation period:	Not specified	
Diet/Food:	Lucerne grass, carrots, germinated grains with wheat bran	
Water:	Not specified	
Housing:	Individual	
Environmental conditions:	Temperature:	not specified
	Humidity:	not specified
	Air changes:	not specified
	Photoperiod:	Regular lighting conditions

B: Study design and methods

In life dates: No information provided

Animal assignment and treatment:

Glyphosate technical was applied topically to shaved skin area of 2 female and 2 male rabbits at a dose level of 2000 mg/kg bw. Additional 2 rabbits per sex served as a control group. A gauze patch was secured over each treated area by means of adhesive tape. After 24-hours the patchs were removed and the test site was washed with warm water.

The animals were observed for 15 days for toxic symptoms and mortality.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Single dermal administration of 2000 mg/kg bw to 2 male and 2 female rabbits did not reveal any clinical signs of toxicity. Slight erythema was observed at the site of treatment for 24-hours.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

No necropsy was conducted.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate technical after single dermal administration to male and female Albino NWS rabbits, observed over a period of 15 days was estimated to be greater than 2000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Due to deviations of the study and the fact that it was not performed according to any guideline and not according to GLP, the study provided supplementary information on acute dermal toxicity of glyphosate, only.
The available study report in pdf format was of poor quality, so that not all information could be finally verified.

Assessment and conclusion by RMS:
Only limited information is given in the study report and moreover the study report has faded and is hard to read. Due to the limited information available, the fact that the study is not guideline compliant and not GLP, the study is considered unacceptable.

B.6.2.2.27. Study 27

Data point	CA 5.2.2/027
Report author	[redacted]
Report year	1981
Report title	Acute dermal toxicity of MON 0139 to rabbits
Report No	800258
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 402, 2017)	No stepwise approach was used, five rabbits of both sexes instead of two animals, preferred female rats, were used per dose. High dose (above suggested limit dose of 2000 mg/kg bw) was selected. The following data are not reported: Purity of the test material, age of animals, environmental conditions, type of diet, acclimation period, area of the skin surface covered with the test material, dosing volume, individual animal data (body weights, signs of toxicity, necropsy findings). Observation time on the day of application not specified as recommended in the test guideline. The skin was abraded with a hypodermic needle prior to test material administration. Instead of a semi-occlusive dressing an occlusive dressing was used. Both the abrasion of the skin and the occlusive dressing can be considered as worst-case.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognized testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered unacceptable

The acute dermal toxicity potential of the test material MON 0139 was investigated following single application to 10 rabbits (five males, five females). The test substance was applied to clipped and abraded skin at a dose of 5000 mg/kg bw. After 24-hours contact period the test substance was removed and the rabbits were observed for 14 days. No deaths were observed in animals of either sex. No clinical signs of toxicity were observed. At necropsy, one male animal had fur stained with diarrheal feces, which was not attributed to toxicity of the test material. The acute dermal LD_{50} was

LD_{50}, dermal, rabbit > 5000 mg/kg bw.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: MON 0139
Description: Amber liquid
Lot/Batch #: SSRT-11012
Purity: Not specified
Stability of test compound: Not specified

2. **Vehicle and/ or positive control:** none
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White (Isf: (NZW)
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Males and females
Weight at dosing:	1.99 – 2.56 kg
Acclimation period:	Not specified
Diet/Food:	Not specified, *ad libitum*
Water:	Not specified, *ad libitum*
Housing:	Individually housed
Environmental conditions:	Temperature: not specified
	Humidity: not specified
	Air changes: not specified
	Photoperiod: not specified

B: Study design and methods

In life dates: 1980-08-25 to 1980-09-08

Animal assignment and treatment:

Five male and five female rabbits were prepared by clipping the skin on the dorsal surface and abrading with a hypodermic needle. MON 0139 was applied topically to the dorsal surface at a dose level of 5000 mg/kg bw. The test material was held in place by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. After 24-hours the occlusive wraps were removed and the excess material was wiped from the animal.

Observations for mortality and clinical signs of toxicity were made three times during the first eight hours following test material administration and twice daily thereafter. For each animal body weight was recorded on the day of exposure (Day 0), on Day 7 and on Day 14. The animals were sacrifice at the end of the 14-day observation period and subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths were observed in animals of either sex.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed in any of the test animals during this study.

C. BODY WEIGHT

Mean body weight gains were acceptable.

D. NECROPSY

One male animal had fur stained with diarrheal feces. This was not attributed to toxicity of the test material. No abnormalities were noted for the remaining nine animals.

III. CONCLUSIONS

The acute dermal LD_{50} of glyphosate salt after a single dermal administration to male and female New Zealand White rabbits, observed over a period of 14 days was greater than 5000 mg/kg bw.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Due to the deviations of the study and the fact that it was not performed according to GLP, the study provides supplementary information on acute dermal toxicity of glyphosate, only.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered unacceptable due to the study not being guideline compliant or GLP, the missing data on the purity of the test substance, the limited reporting and missing individual data and the worst-case study design by abrading the skin and using occlusive dressing and thus no conclusion can be drawn.

B.6.2.3. Inhalation

B.6.2.3.1. Study 1

Data point:	CA 5.2.3/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat
Report No	11/054-004P
Document No	Not reported
Guidelines followed in study	OECD 403 (2009): OPPTS 870.1300 (1998): 440/2008 B.2 (2008
Deviations from current test guideline	The temperature and humidity value deviated from required range during the animal exposure. These deviations did not affect the study outcome.
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

In an acute inhalation toxicity study, a group of young adult Wistar RjHan: (WI) strain rats, (5 males and 5 females) was exposed to a target aerosol concentration of 5 mg/L glyphosate technical (96.9 % w/w glyphosate technical). The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14-day observation period. The day of exposure was designated Day 0. Aerosol concentrations were measured gravimetrically. The particle size distribution of the test aerosol was determined regularly during the exposure period.

Clinical observations and bodyweights were recorded throughout the study and at the end of the scheduled period the animals were killed and subjected to a gross examination *post mortem*.

The mean achieved atmosphere concentration was 5.04 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was 3.65 µm ± 2.24 (GSD [Geometric Standard Deviation]).

One male rat died following a 4-hour exposure to 5.04 mg/L Glyphosate Technical on Day 4.

Wet fur and fur staining were commonly recorded on the day of exposure and on the day after exposure. These observations were considered to be related to the restraint and exposure. Significant clinical signs were recorded on the day of exposure and the following day included laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, activity decreased, thin body appearance (weak/wasted). The majority of the animals recovered from Day 3.

Normal bodyweight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during the first week of the observation period.

No macroscopic findings were seen at necropsy. A specific cause of death was not determined for the single male that died.

Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 5.04 mg/L for 4 hours.

The acute LC_{50} of Glyphosate Technical in rats is therefore considered to be greater than 5.04 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	Technical; dry white powder
Lot/Batch #:	614034 (20100609\Milled)
Purity:	96.9 % w/w Glyphosate technical
Stability of test compound:	Stable under storage conditions (room temperature range <30 °C), recertification date end January 2014

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar RjHan:WI
Source:	[redacted]
Age:	8 - 10 weeks
Sex:	Males and females
Weight at dosing:	229 – 386 g
Acclimation period:	At least five days
Diet/Food:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany, *ad libitum* (except during exposure)
Water:	Tap water, *ad libitum* (except during exposure)
Housing:	In groups of 5 (or 2 in the case of sighting exposure), by sex, in solid-floor cages (Type III) with stainless steel mesh lids and softwood flake bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 – 70 % Air changes: 15 – 20 air changes per hour Photoperiod: 12 hours light / 12 hours dark

B. STUDY DESIGN AND METHODS

In life dates: 2011-04-14 to 2011-04-28

Exposure conditions: Prior to animal exposures, test material atmospheres were generated within the exposure chamber. During these technical trials, air-flow settings and test material input rates were adjusted to achieve the required atmospheric characteristics. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of Glyphosate Technical.

Exposure system: The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of 2, concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.

Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nares to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic.

Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least 0.7 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.

Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones.

Exposure procedure: Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber. Only the nose of each animal was exposed to the test atmosphere. Following an equilibration period of at least the theoretical chamber equilibration time (T99), a group of 10 rats (5 male and 5 female) was exposed to a target atmosphere concentration 5 mg/L for a period of at least 4 hours.

Generation of the test atmosphere / chamber description: The test item was aerosolised using a rotating brush powder disperser (Palas GmbH, Karlsruhe, Germany) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.

Test atmosphere concentration: The test atmosphere was sampled at regular intervals during each exposure period. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters. The difference in the pre and post sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.

The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that through the chamber during the same period.

Particle size determination: The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (which employs an inertial separation technique to isolate particles in the discrete aerodynamic size ranges). Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).

The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.
The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 μm was calculated.

From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation were calculated. In addition, the proportion (%) of aerosol less than 4 µm (considered to be the inhalable portion) was determined.

Table 6.2.3.1-1: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat [redacted] 2011): Summary of main study test atmosphere characteristics

Parameter	Target concentration 5 mg/L	
Mean achieved concentration (mg/L)	5.04 ± 0.17	
Nominal (mg/L)	7.71	
Particle size MMAD; GSD	3.65 µm; 2,24	
Inhalable fraction (% < 4 µm)	54.4	
	% by weight in range	
Size range (µm)	Total mass/stage (mg)	Cumulative mass (%)
<0.55	0.35	2.05
0.55 – 0.96	0.30	3.81
0.96 – 1.55	0.91	9.13
1.55 – 2.11	1.90	20.26
2.11 – 3.56	5.43	52.05
3.56 – 6.66	4.69	79.51
6.66 – 10.55	2.06	91.57
>10.55	1.44	100.00
T99 (Minimum Acceptable Equilibration Time)	1 minute	
Chamber volume (inner plenum)	3.85 L	
Air Flow In (Inner Plenum) (L/min)	20.0 - 20.6	
Air Flow Out (Inner Plenum) (L/min)	19.4 – 38.4	
Temperature	21.6 – 24.7 °C	
Humidity	3.9 – 10.2 % (n=3)	
Oxygen Concentration (%)	19.6 – 20.3	
Carbon Dioxide	0.1 – 0.8	

Sighting studies: Two sighting exposures using 2 male and 2 female rats were performed before the main study due to insufficient information about the test item's inhalation toxicity.

Animal assignment and treatment: Five male and 5 females were exposed to a target aerosol concentration of 5 mg/L Glyphosate Technical. The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14 day observation period. The day of exposure was designated Day 0.

Animals were checked hourly during exposure, 1 hour after exposure and twice daily (early and late in the working day) during the 14 days of the observation period for morbidity and/or mortality. All animals were observed for clinical signs at hourly intervals during exposure, as soon as practically possible following removal from restraint at the end of exposure, 1 hour after exposure and subsequently once daily for 14 days. The body weight of each rat was recorded prior to treatment on the day of exposure (day 0) and on Days 1, 3, 7 and 14.

At the end of the 14 day observation period, the animals were sacrificed by exsanguination under anaesthesia and a gross macroscopic examination was performed, which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.

Statistics: The acute inhalation LC_{50} was calculated from the mortality data.

II. RESULTS

A. MORTALITY

One male rat died on Day 4 following a 4-hour exposure to 5.04 mg/L glyphosate technical.

B. CLINICAL OBSERVATIONS

Wet fur and fur staining were commonly recorded on the day of and the day following exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be treatment related.

Significant clinical signs were recorded on day of exposure and the following day included laboured and noisy respiration, respiratory rate increased, gasping respiration, sneezing, decreased activity, thin body appearance (weak/wasted).

The majority of animals recovered from Day 3.

The clinical signs are summarized in the table below.

Table 6.2.3.1-2: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat [redacted] 2011): Clinical observation data

Clinical sign	Sex	No. of animals exposed [1]	Hours During exposure			On removal from chamber (4 hours)	One-hour Post-exposure	Post-exposure Day				
			1	2	3			1	2	3	4	5-14
Wet fur (on/in restraining apparatus)	Male	5	2	5	5	0	0	0	0	0	0	0
	Female	5	3	5	5	0	0	0	0	0	0	0
Wet fur (whole body)	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0
Ruffled coat	Male	5	0	0	0	0	0	2	0	0	0	0
	Female	5	0	0	0	0	0	1	0	0	0	0
Laboured respiration	Male	5	2	3	4	5	5	$3^{2,4}$	1^{4}	1^{4}	0	0
	Female	5	2	2	4	4	4	1	0	0	0	0
Noisy respiration	Male	5	0	0	0	1	1	2^{2}	1^{4}	1^{4}	0	0
	Female	5	0	0	0	1	1	0	0	0	0	0
Respiratory rate	Male	5	0	0	0	0	0	0	0	0	0	0

increased	Female	5	0	0	1	2	2	0	0	0	0	0
Gasping respiration	Male	5	0	0	0	0	0	1[4]	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Sneezing	Male	5	0	0	0	0	0	1	1	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Red-Brown staining (Head, Carnium)	Male	5	0	0	0	5	5	1	0	0	0	0
	Female	5	0	0	0	1	1	0	0	0	0	0
Red-Brown staining (Snout)	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0
Red-Brown staining (Nose)	Male	5	0	0	0	0	0	5	1[4]	0	0	0
	Female	5	0	0	0	0	0	3	0	0	0	0
Activity decreased	Male	5	0	0	0	0	0	1[4]	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Weak	Male	5	0	0	0	0	0	3[3]	3[3]	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Wasted	Male	5	0	0	0	0	0	0	0	1[4]	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0

[1] Day 4 after exposure: 1 male was found dead
[2] One male with severe symptom
[3] One male moderate weak and two males slightly weak
[4] Male found dead on Day 4

C. BODY WEIGHT

Normal body weight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during first week of the observation period.

The body weight data are summarized in the table below.

Table 6.2.3.1-3: Glyphosate technical: Acute inhalation toxicity study (nose-only) in the rat [redacted] 2011): Body weight data

Main study		Day 0	Day 1	Day 3	Day 7	Day 14
Male	Mean body weight (kg)	379	354	362	395	432
	Standard deviation	7.8	10.5	20.8	19.4	13.0
Female	Mean body weight (kg)	232	223	234	247	261
	Standard deviation	2.3	4.3	2.3	4.4	5.4

Note: Mean and standard deviation were not given in the study report. Values were calculated retrospectively based on individual animal data.

D. NECROPSY

There were no macroscopic abnormalities in animals surviving to scheduled termination. A specific cause of death was not determined for the single male that died in the main study. The pathology report for the male that died in the main study reported dark/red discoloration of the lungs and thymus.

III. CONCLUSIONS

Under the experimental conditions of this study, a single death occurred in a group of 10 rats exposed to glyphosate technical to a mean achieved atmosphere of 5.04 mg/L for 4 hours. The acute inhalation LC_{50} of glyphosate technical, after a 4-hour exposure to male and female Wistar RjHan: (WI) strain rats, observed over a period of 14 days is considered to be greater than 5.04 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current OECD 403 guideline (2009) and according to GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5.04 mg/L air. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the acute inhalation LC_{50} >5.04 mg/L air in male and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.3.2. Study 2

Data point:	CA 5.2.3/002
Report author	[redacted]
Report year	2010
Report title	Acute Inhalation Toxicity Study of Glyphosate TC In Rats
Report No	24603
Document No	Not reported
Guidelines followed in study	EC method B.2, OECD 403 (1981), EPA Health Effects Test Guidelines, OPPTS 870.1300
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (4.633 µm with a GSD of 3.02 (no smaller MMAD and GSD could be obtained with the test item supplied according to the study author)). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Due to the deviations noted above, the study is considered acceptable, but with restrictions.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in rats when administered for a single 4-hour period using a dynamic nose-only exposure chamber at an actual concentration of 5.18 mg/L.

No mortality occurred during the study. Clinical signs included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings.

The acute inhalation LC_{50} was determined to be > 5.18 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3%
Stability of test compound:	At room temperature in the dark stable until May 2011

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rat
Strain:	CD/Crl:CD (SD)
Source:	[redacted]
Age:	approx. 7 – 9 weeks
Sex:	Males and females
Weight at dosing:	♂ 234 – 270 g; ♀ 208 – 244 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff RIM-H V1 534 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* (except 16 h before exposure)
Water:	tap water, *ad libitum*
Housing:	In groups of 2 – 3 animals per cage in Makrolon type III plus cages with granulated textured wood bedding.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15% Air changes: no data 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2010-02-02 to 2010-02-16

Test atmosphere generation:

A dust atmosphere was produced from the test material using a rotating brush dust generator and compressed air.

Exposure chamber conditions:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals. The cylindrical exposure chamber had a volume of approximately 40 L. The actual dust concentration was measured four times gravimetrically with an air sample filter (Minisart SM 17598; 0.45 pm) and pump (Vacuubrand, MZ 2C, Vacuubrand, Germany) controlled by a rotameter. Dust samples were taken once every hour during the exposure. For that purpose, a probe was placed close to the animals' noses in the inhalation chamber and air was sucked through the air sample filter at a constant flow of air of 5 L/min for 1 minute. The filters were weighed before and after sampling on an analytical balance (accuracy 0.1 mg).
Chamber airflow rates ranged from 800 to 900 L/h, providing ≥ 12 air changes per hour.

Particle size distribution:

A Malvern Spraytec Lasersystem (Malvern Instruments, Germany) was employed for the determination of the particle size distribution of the particle diameter (volume) in the exposure air. The particle size distribution of the test atmospheres was measured using a cascade impactor two times during the exposure period. The results were as follows:

Table 6.2.3.2-1: Acute Inhalation Toxicity Study of Glyphosate TC In Rats [redacted] 2010): Details of test atmosphere

Mean achieved actual concentration (HPLC)	Actual concentration (gravimetric method)	MMAD	GSD	Respirable amount particle size ≤4 µm	
(mg/L air)	(mg/L air)	(µm)		(mg/L air)	(%)
5.18	5.05	4.633	3.02	1.08	20.8

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 4.633 µm as determined with a cascade impactor. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 3.02. No smaller MMAD and GSD could be obtained with the test item supplied.

Animal assignment and treatment:

A group of five fasted rats per sex received the test material at a dose level of 5.18 mg/L using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure. Observations for mortality and clinical/behavioural signs of toxicity were made at least once per day for 14 days. Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure. The clinical signs are summarized in the table below.

Table 6.2.3.2-2: Acute Inhalation Toxicity Study of Glyphosate TC In Rats [redacted] 2010): Clinical observation data

Test days			**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Time after administration (in minutes unless otherwise stated)			**0**	**5**	**15**	**30**	**60**	**3 h**													
Sex		Clinical signs	Animals affected/investigated																		
M		tremor	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F		tremor	5/5	5/5	5/5	5/5	5/5	5/5	-	-	-	-	-	-	-	-	-	-	-	-	-
		dyspnoea	5/5	5/5	5/5	5/5	5/5	5/5	-	-	-	-	-	-	-	-	-	-	-	-	-

C. BODY WEIGHT

All animals gained the expected body weight.

D. NECROPSY

No pathological findings were noted at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.18 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and GSD slightly exceed the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Due to the deviations mentioned above the study is considered acceptable, but with restrictions. Based on the study results, the acute inhalation LC_{50} is >5.18 mg/L air after an exposure period of 4 hours. The study was accepted in the previous evaluation (RAR, 2015).

B.6.2.3.3. Study 3

Data point:	CA 5.2.3/003
Report author	[redacted]
Report year	2010
Report title	Acute Inhalation Toxicity Study of Glyphosate TC in Rats
Report No	24875
Document No	Not reported
Guidelines followed in study	EC method B.2. (92/69/EEC), OECD 403 (1981) and OPPTS 870.1300
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (4.197 µm with a GSD of 2.64 (no smaller MMAD and GSD could be obtained with the test item supplied)). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Due to the deviations noted above, the study is acceptable, but with restrictions.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined by HPLC, was 5.02 mg/L air for 4 hours. In the inhalation chamber, close to the animals' noses, the generated dust had a mass median aerodynamic diameter (MMAD) of 4.197 ± 2.64 µm as determined with a cascade impactor. No smaller MMAD could be obtained with the test item according to the study author. The analysis of the particle size distribution of the particle diameter (volume) in the exposure air was carried out by laser measurement and determined as $d_{[50]}$ = 37.15 µm. The particle size distribution of the particle size of the delivered test item was $d_{[50]}$ = 14.5 µm. The test concentration revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be > 5.02 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	May 2011

2. **Test animals:**

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: approx. 7 weeks Females: approx. 9 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 270 – 282 g; Females: 220 – 251 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70 % Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-10-15 to 2010-02-18

Animal assignment and treatment:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15 minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. Animals were exposed for 4 hours to an actual concentration of 5.02 mg/L air (determined by HPLC).

A laser measured the size of the individual particles or individual aerosol drops. The particle size distribution for the estimation of the Mass Median Aerodynamic Diameter (MMAD) was carried out twice during the exposure period using a cascade impactor. The median particle size distribution of the test item was determined with a Malvern Sizer.

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The actual dust concentration of 5.02 mg Glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of Glyphosate TC was as follows:

Table 6.2.3.3-1: Acute Inhalation Toxicity Study of Glyphosate TC in Rats [redacted] 2010): Details of test atmosphere

Actual concentration (HPLC) [mg/L air]	Actual concentration (gravimetric method) [mg/L air]	MMAD [µm]	Respirable amount particle size ≤4 µm	
			[mg/L air]	[%]
5.02	4.99	4.197 ± 2.64	1.03	20.5

No smaller MMAD could be obtained with the test item and no higher fraction of respirable particles could be obtained.

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration 5.02 mg/L air
$d_{[10]}$	12.51 µm
$d_{[50]}$	37.15 µm
$d_{[90]}$	86.42 µm

[xx] = percentage of cumulative particle size distribution

The particle size distribution of the delivered test item was $d_{[50]}$ = 14.5 µm.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

A 4-hour exposure to Glyphosate TC at the concentration of 5.02 mg/L revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure. The clinical signs are summarized in the table below.

Table 6.2.3.3-2: Acute Inhalation Toxicity Study of Glyphosate TC in Rats [redacted] 2010): Clinical observation data

Test days			**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Time after administration (in minutes unless otherwise stated)			**0**	**5**	**15**	**30**	**60**	**3h**													
Sex		Clinical signs	Animals affected/investigated																		
M		ataxia	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		tremor	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F		ataxia	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		tremor	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to males and females rats, observed over a period of 14 days was greater than 5.02 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and GSD slightly exceed the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valide OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC50 is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Due to the deviations mentioned above the study is considered acceptable, but with restrictions. The LC_{50} is >5.02 mg/L air in males and females.
The study was accepted in the previous evaluation (RAR, 2015).

B.6.2.3.4. Study 4

Data point:	CA 5.2.3/004
Report author	[redacted]
Report year	2009
Report title	Acute Inhalation Toxicity Study of Glyphosate TC in Rats
Report No	23911
Document No	Not reported
Guidelines followed in study	EC method B.2. (92/69/EEC), OECD 403 (1981) and OPPTS 870.1300
Deviations from current test guideline (OECD 403, 2009)	No MMAD calculated (mean measured particle size: 6.62 µm). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week. There were several minor deviations from the Study Plan which did not affect the scientific outcome or the validity of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Due to the deviations noted above, the study is considered acceptable, but with restrictions.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined by HPLC, was 5.12 mg/L air for 4 hours. The analysis of the particle size distribution was carried out by laser measurement and determined as $d_{[50]}$ = 6.62 µm. No finer dust concentration of the test item could be generated. The test concentration revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be > 5.12 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8%
Stability of test compound:	2010-08-01

2. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	[redacted]
Age:	Males: 52 days Females: 66 days
Sex:	5 male and 5 female
Weight at dosing:	Males: 240 – 267 g; Females: 209 – 216 g
Acclimation period:	5 days
Diet/Food:	ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
Housing:	Animals were kept by sex in groups of 2-3 animals in MAKROLON cages (type III plus) with granulated textured wood as bedding material.
Environmental conditions:	Temperature: 22 ± 3 °C Rel. humidity: 40 – 70% Air changes: 12/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-02-04 to 2009-07-30

Animal assignment and treatment:

The study was carried out using a dynamic inhalation apparatus (≥ 12 air changes/h) with a nose-only exposure of the animals (exposure chamber volume 40 L). The test item was micronized before administration and the dust was generated with a rotating brush dust generator. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber after equilibration of the chamber concentration for at least 15

minutes. The dust concentration in the inhalation chamber was determined gravimetrically as well as by HPLC once every hour during exposure. A laser measured the size of the individual particles or individual aerosol drops. Animals were exposed four 4 hours to an actual concentration of 5.12 mg/L air (determined by HPLC).

After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made at least once daily until symptoms subsided, and thereafter each working day. Observations on mortality were made at least once daily. Individual body weights were determined before the exposure and weekly after exposure. On Day 14 after completion of exposure, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The actual dust concentration of 5.12 mg Glyphosate TC/L air was measured at the animals' nose and was determined by HPLC. The mean actual exposure concentration of Glyphosate TC was as follows (see table below):

Table 6.2.3.4-1: Acute Inhalation Toxicity Study of Glyphosate TC in Rats [redacted] 2009): Details of test atmosphere

Nominal concentration (test item/water) [mg/L air]	Actual concentration (HPLC) [mg/L air]	median diameter [µm]	Respirable amount particle size ≤3.98 µm	
			[mg/L air]	[%]
5.0	5.12	6.62	< 0.01	< 0.01

Laser measurement revealed the following particle size distribution during the exposure:

Diameter	Actual concentration 5.12 mg/L air
$d_{[10]}$	5.64 µm
$d_{[50]}$	6.62 µm
$d_{[90]}$	8.10 µm

[xx] = percentage of cumulative particle size distribution

No finer dust concentration of the test item could be generated.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

A 4-hour exposure to Glyphosate TC at the concentration of 5.12 mg/L revealed slight dyspnoea and ataxia in all 5 of 5 male and 5 of 5 female animals immediately until 60 minutes after the end of exposure.
The clinical signs are summarized in the table below.

Table 6.2.3.4-1: Acute Inhalation Toxicity Study of Glyphosate TC in Rats [redacted] 2009): Clinical observation data

Test days			**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Time after administration (in minutes unless otherwise stated)			**0**	**5**	**15**	**30**	**60**	**3 h**													
Sex		Clinical signs	Animals affected/investigated																		
M		ataxia	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F		ataxia	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greated than 5.12 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The particle size exceeds the recommended values of the current OECD guideline; however, no finer dust could be generated. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Due to the deviations mentioned above the study is considered acceptable, but with restrictions. The LC_{50} is >5.12 mg/L air in males and females.
The study was accepted in the previous evaluation (RAR, 2015).

B.6.2.3.5. Study 5

Data point:	CA 5.2.3/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat
Report No	2743/0001
Document No	Not reported
Guidelines followed in study	OECD 403 (1981) Commission Regulation (EC) No 440/2008 (2008), method B.2 (2008)
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (5.25 µm with a GSD of 3.35 (due to technical limitations)). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week. The bodyweight of the female animals at dosing was slightly outside the protocol range of 200 -350 g.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered acceptable (reliable with restrictions) since the MMAD and GSD are outside the range recommended in the OECD guidance

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in male and female HsdRccHan™: WIST rats by exposure to the dose level of 5.04 mg/L via dust atmosphere for 4 hours using a nose-only exposure system. No mortality occurred during the study. Clinical signs included increased respiratory rate, hunched posture, pilo-erection and wet fur. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

The acute inhalation LC_{50} was determined to be > 5.04 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate Tech
Description:	White powder
Lot/Batch #:	GI-1045
Purity:	96.66%
Stability of test compound:	Expiration in July 2010

2. **Vehicle and/or positive control:** Not relevant

3. Test animals:

Species:	Rat
Strain:	HsdRccHan™ : WIST
Source:	[redacted]
Age:	Approx. 8 - 12 weeks
Sex:	Male and female
Weight at dosing:	178 – 350 g
Acclimation period:	5 days
Diet/Food:	With the exception of the exposure period, free access to food (Harlan 2014 Rodent Diet, Harlan UK Limited, Oxon, UK) was allowed throughout the study
Water:	With the exception of the exposure period, free access to drinking water was allowed throughout the study
Housing:	Housed in groups of five by sex in solid-floor polupropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd., Cheshire, UK) and provided with environmental enrichment items: wooden chew blocks and cardboard "fun tunnels" (Datesand Ltd., Cheshire, UK)
Environmental conditions:	Temperature: 19 – 25 °C Humidity: 30 – 70% Air changes: At least 15/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2009-05-12 to 2009-06-04

Animal assignment and treatment:

Five male and female rats were exposed to one dose level of dust atmosphere of glyphosate. The single 5 mg/L four-hour exposure was "nose only" at a mean actual concentration of 5.04 ± 0.37 mg/L (nominal concentration was 27.3 mg/L).

Operational conditions (flow rate, oxygen levels, temperature, and humidity in the inhalation systems) were checked throughout the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for 14 days. Individual body weights were recorded prior to treatment on the day of exposure and on Days 7 and 14. At the end of the fourteen-day observation period the animals were killed by intravenous overdose of sodium pentobarbitone. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.

The chamber flow rate was maintained at 45 L/min providing 90 air changes per hour.

II. RESULTS

A. TEST ATMOSPHERE

The particle size analysis of the atmosphere drawn from the animals' breathing zone, was as follows:

Table 6.2.3.5-2: Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat [redacted] 2009): Details of test atmosphere

Mean Achieved Atmoshpere Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (μm)	Inhalable Fraction (% <4 μm)	Geometric Standard Deviation
5.04	5.25	41.1	3.35

It is noted that the achieved particle size distribution is outside of the range required by the test guidelines.

During characterisation, changes were made to the generation system (addition of particle sizes separator) and grinding techniques in an attempt to increase the inhalable portion of the test material. However, this reduced the achieved concentration, and therefore, also reduced the actual concentration of particles <4 μm. It was, therefore, preferable to expose the animals to a higher concentration of test material, even though this also increased the mean mass median aerodynamic diameter, as this resulted in the animals being exposed to the highest possible concentration of particles <4 μm.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

Signs of hunched posture and piloerection are commonly seen in animals for short periods on removal from the chamber following 4-hour inhalation studies. Wet fur is commonly recorded both during and for a short period after exposure. These observations are considered to be associated with the restraint procedure and, in isolation, are not indicative of toxicity.

In addition to the observations considered to be due to the restraint procedure, increased respiratory rate was noted in all animals during exposure, on removal from the chamber and one-hour post-exposure.
The clinical signs are summarized in the table below.

Table 6.2.3.5-3: Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat [redacted] 2009): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day					Post-exposure Day							
			During exposure (h)			On removal from chamber	Post-exposure (h)								
			1	2	3		1	1	2	3	4	5	6	7	8-14
Hunched posture	Male	5	0	0	0	5	5	0	0	0	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0	0	0	0

Pilo-erection	Male	5	0	0	0	5	5	0	0	0	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0	0	0	0
Increased respiratory rate	Male	5	5	5	5	5	5	0	0	0	0	0	0	0	0
	Female	5	5	5	5	5	5	0	0	0	0	0	0	0	0
Wet fur	Male	5	5	5	5	5	5	0	0	0	0	0	0	0	0
	Female	5	5	5	5	5	5	0	0	0	0	0	0	0	0

D. BODY WEIGHT

Normal body weight development was noted during the course of the study.

E. NECROPSY

No macroscopic abnormalities were detected at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.04 mg/L.

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and GSD slightly exceed the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC50 is >5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
The mass median aerodynamic diameter and geometric standard deviation of the particle size distribution are outside the range required by the guidance document. Therefore, the study is considered acceptable but with restrictions also considering that there are other studies available that are fully guideline compliant. The study confirms the acute inhalation LC_{50} > 5 mg/L air in male and female rats.

B.6.2.3.6. Study 6

Data point:	CA 5.2.3/006
Report author	[redacted]

Report year	2009
Report title	Glyphosate – Acute Inhalation Toxicity Study in Rats
Report No	12107-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.1300.
Deviations from current test guideline (OECD 403, 2009)	Humidity was in the range of 33 – 89% instead of 30 – 70%. Female weight was outside the protocol range. Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. The geometric standard deviation was not calculated. The tested concentration is considered low for a limit test and the study report does not describe attempts to increase the concentration tested while maintaining a respirable size distribution. Therefore, the study is considered reliable with restrictions
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 (reliable with restrictions) due to the low concentration tested.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. Five male and five female rats were exposed for 4 hours to an aerosol generated from the undiluted test substance at a level of 2.24 mg/L. The exposure concentration was determined gravimetrically. The analysis of the particle size distribution was carried out by a cascade impactor and the mass median aerodynamic diameter (MMAD) was estimated to be 2.6 µm. There was no mortality during the study. Clinical sings included piloerection and activity decrease, which were no longer evident by Day 4. Body weights were unaffected by the exposure. The gross necropsy revealed no observable abnormalities.

The acute inhalation LC_{50} was determined to be > 2.24 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Tech Grade Mixed 5-Batch

Description: White powder

Lot/Batch #: 080704-1 thru 5

Purity: 96.71 % (analysed 2009-01-08); 96.40 % (analysed 2008-10-17)

Stability of test compound: No data given in the report.

2. Test animals:

Species: Rat albino

Strain / Stock: Sprague-Dawley

Source: [redacted]

Age: Approx. 7 – 8 weeks

Sex: 5 male and 5 female

Weight at dosing: Males: 262 – 289 g; Females: 172 – 191 g

Acclimation period:	5 days
Diet/Food:	Formula #5008 (PMI Feeds Inc.), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individual housing in suspended, wire bottom, stainless steel cages.
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 30 – 70 %
	Air changes: 10 – 12/hour
	12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2008-11-07 to 2008-11-21

Animal assignment and treatment:

The study was carried out using a 500 L nose-only stainless steel, dynamic flow inhalation chamber with 25 ports in 5 rows. Polycarbonate tubes were inserted into 10 designated individual ports. The test substance was ground for 10 hours and dried prior to exposure. The aerosol was generated from the undiluted test substance by a Venturi Aspirator and sprayed directly into the exposure chamber. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol for a period of 4 hours. The dust concentration in the inhalation chamber was determined gravimetrically twice per hour and nominally at the end of the exposure. Particle size, taken from the breathing zone of the animals, was determined twice during the exposure using a cascade impactor, and the mass median aerodynamic diameter (MMAD) and particle size distribution were calculated.

Observations for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to the inhalation exposure and on Days 7 and 14. On Day 14 after completion of exposure, all animals were euthanized by an intraperitoneal injection, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The exposure concentration was determined to be 2.24 mg/L with an average MMAD of 2.6 µm.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

The only prominent in life observations were piloerection and activity decrease. Animals were asymptomatic by Day 4. The clinical signs are summarized in the table below.

Table 6.2.3.6-4: Glyphosate – Acute Inhalation Toxicity Study in Rats [redacted] 2009): Clinical observation data

Clinical sign	Sex	No. of animals	Exposure Day	Post-exposure Day

		exposed	Time after exposure begins (h)												
			0.5	1.0	2.5	4.5	6.0	1	2	3	4	5	6	7	8-14
Pilloerection	Male	5	0	0	0	5	5	5	5	0	0	0	0	0	0
	Female	5	0	0	0	5	5	5	5	0	0	0	0	0	0
Activity decrease	Male	5	0	0	0	5	5	5	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	5	5	5	0	0	0	0	0

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 2.24 mg/L air.

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC50 is > 2.24 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered acceptable, but with restrictions. The tested concentration is considered low for a limit test and the study report does not describe attempts to increase the concentration tested while maintaining a respirable size distribution. Also, the geometric standard deviation was not calculated. Under the conditions of the study the acute inhalation LC_{50} > 2.24 mg/L air in male and female rats

B.6.2.3.7. Study 7

Data point:	CA 5.2.3/007
Report author	[redacted]
Report year	2008
Report title	Acute Inhalation Toxicity Test of Glyphosate Technical in Rats *(Rattus norvegicus)*
Report No	[redacted]3996.309.377.07
Document No	Not reported

Guidelines followed in study	OECD guideline 403 (1981)
Deviations from current test guideline (OECD 403, 2009)	MMAD exceeded the recommended MMAD (MMAD ranged from 18.555 to 19.901 µm, only 4.72 to 5.15 % of the particles with an aerodynamic diameter <3.42 µm were within the respirable size range; no explanation provided why the standard could not be achieved). Experimental phase initiation and conclusion dates were updated. Limit test performed on 5 animals per sex.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Invalid, Category 3b **Conclusion AGG:** The study is considered unacceptable due to small amount of particles that were within the respirable range.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. One group of rats (five/sex) was exposed nose-only for a 4-hour exposure period to the aerosolized test item, using a total airflow of 10 L/min. The aerodynamic particle size distribution determined with a cascade impactor indicated that 4.72 to 5.15 % of the aerosol generated was within the respirable size range. The mass median aerodynamic diameter (MMAD) ranged from 18.555 to 19.901 µm. The mean actual concentration determined gravimetrically was 5.211 mg/L. No mortality occurred during the study and no pathological findings were noted at necropsy. Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period. All animals gained the expected body weight, except for the males on the first post-exposure day.

The acute inhalation LC_{50} was determined to be > 5.211 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate Technical

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report.

2. **Test animals:**

Species:	Rat albino (*Rattus norvegicus*)
Strain / Stock:	Wistar Hannover
Source:	[redacted]
Age:	Males: 9 weeks; Females: 11 weeks
Sex:	5 males and 5 females
Weight at dosing:	Males: 262 – 291 g; Females: 178 – 208
Acclimation period:	9 days
Diet/Food:	Nuvilab CR-1 pellet diet type for rodents (Nuvital Nutrients Ltda.), *ad libitum*
Water:	Potable drinking water, *ad libitum*

Housing:	Polypropylene rodents cages with autoclaved wood shavings and stainless steel mesh lids containing five rats of each sex per cage.
Environmental conditions:	Temperature: 19 – 25 °C
	Humidity: 30 – 70 %
	Air changes: 10 – 15/hour
	12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2008-06-06 to 2008-06-20

Animal assignment and treatment:

The study was carried out using an inhalation chamber with a nose-only exposure of the animals. The test item was aerosolized. Exposure started by locating the rats (5 male and 5 female animals) into the exposure chamber. Animals were exposed to the aerosol at the maximum attainable concentration (5.211 mg/L) for a period of 4 hours. The actual concentration in the inhalation chamber was determined gravimetrically by taking eight equally time-spaced air samples from the breathing zone. Aerodynamic particle size distribution was determined two times using a Seven Stage Cascade Impactor.
After completion of exposure, animals were observed for a period of 14 days. Observations for clinical/behavioural signs of toxicity were made right after the exposure, and thereafter each working day. On Day 14 after completion of exposure, all animals were euthanized in a carbon dioxide chamber, dissected and inspected macroscopically. All gross pathological changes were recorded. No microscopic examination was performed as no pathological findings were noted at necropsy.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The mean actual concentration was 5.211 mg/L. The actual concentration of the test item in each sample was within the ±15 % interval from the mean actual concentration, indicating that the test atmosphere was held stable over the 4-hour exposure period.

Analysis of the particle size distribution of samples from the breathing zone indicates that 4.72 to 5.15 % of the mass collected from the aerosol were within the respirable size range. The MMAD ranged from 18.555 to 19.901 µm and the geometric standard deviation (GSD) ranged from 2.869 to 2.914.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

Clinical signs observed during the 14-day observation period included wheeze and dyspnoea. These acute respiratory signs started within the first day and reverted within the fourth day of the observation period.

The clinical signs are summarized in the table below.

Table 6.2.3.7-5: Acute Inhalation Toxicity Test of Glyphosate Technical in Rats *(Rattus norvegicus)* [redacted] 2008): Clinical observation data

Test days			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex		Clinical signs	Animals affected/investaged														
M		wheeze	1/5	1/5	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	3/5	3/5	3/5	3/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
F		wheeze	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		dyspnoea	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5

D. BODY WEIGHT

The mean body weight increased for both sexes, except for the males on the first post-exposure day (-3.22%). All animals exceeded their initial body weight by the conclusion of the experimental phase.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.211 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is not acceptable as the MMAD is far above the recommended values according to OECD 403 guideline. The MMAD ranged from 18.555 to 19.901 µm.

Assessment and conclusion by RMS:
The study is compliant with the 1981 OECD guideline 403, where no requirements for the MMAD and GSD were set. However, it is agreed with the previous evaluation (RAR, 2015) that the study is unacceptable since only 4.72 to 5.15% of the particles were within the respirable size range as the MMAD ranged from 18.555 to 19.901 µm. Therefore, no conclusion can be drawn.

B.6.2.3.8. Study 8

Data point:	CA 5.2.3/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats
Report No	B02327
Document No	Not reported
Guidelines followed in study	European Communities, Directive 92/69/EEC, Part B.2 "Acute Toxicity (Inhalation)", published December 29, 1992. - OECD Guidelines for Testing of Chemicals, Section 4, No. 403: "Acute Inhalation Toxicity", adopted May 12, 1981. - U.S. Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.1300, Acute Inhalation Toxicity, August 1998. - Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute Inhalation Toxicity

	Studies Guideline 2-1-3. Notification 12 NohSan No. 8147, as partly revised in 16-Shouan-9260, on 16 March 2005. English translation by ACIS on 17 Oct 2005
Deviations from current test guideline (OECD 403, 2009)	The reference to the JMAFF inhalation test guideline was altered on request of the Sponsor. However, this did not affect the contents of the JMAFF inhalation test guideline. In the animal room, on brief occasions (for a total of less than 2 hours) the relative humidity was slightly higher than the upper limit of the target range given in the study plan. On the day of inhalation exposure (test day 1), the total aerosol generation period lasted 4 hours and 30 minutes, because a test aerosol was generated also for 30 minutes prior to the beginning of the exposure. This 30-minute pre-exposure aerosol generation period was used for fine-tuning of the settings of the aerosol generation and exposure system for the inhalation exposure. Consequently the nominal test atmosphere concentration was determined for the total of 4 hours and 30 minutes of aerosol generation (30 min pre-exposure aerosol generation without animals being present plus 4 h inhalation exposure of the animals). Limit test performed on 5 animals per sex. The minor deviations from the study plan were considered not to have compromised the quality, integrity or outcome of the study
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

A group of five male and five female albino rats [HanRcc:WIST(SPF)] was exposed by nose-only, flow-past inhalation to glyphosate technical (NUP 05068) at a gravimetrically determined mean aerosol concentration of 3.252 mg/L air (s.d. ± 0.053, n = 4). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

Two gravimetric measurements of particle size distribution during the exposure produced mass median aerodynamic diameters and geometric standard deviations (GSD) of 2.95 µm (GSD 2.97) and 3.05 µm (GSD 2.73). All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. over a 15-day observation period. Body weights were recorded prior to exposure on test day 1, and during the observation period on test days 4, 8 and 15. On day 15, all animals were sacrificed and necropsied. The ranges of temperature, relative humidity, oxygen content, particle size and airflow measured during the exposure were considered to be satisfactory for a study of this type. There were no deaths and no macroscopic pathology findings. Clinical signs consisted of salivation in two male animals, and transient effects on breathing, i.e. deep respiration and/or rattling breath sounds, in these two and another male, as well as two female animals. Two days after the exposure (test day 3) until the scheduled necropsy day (test day 15) all animals were free from clinical signs. Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals. The clinical signs and the transient losses in body weight were attributed to the treatment with the test item, although slight physical stress during restraint in the exposure tubes may have contributed to the effect on body weight.

In conclusion, the LC_{50} of glyphosate technical (NUP 05068) obtained in this study was estimated to be greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1%
Stability of test compound:	Stable under storage conditions.

2. **Vehicle and/ or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted]
Age:	Males: 9 weeks; Females: 10 weeks
Sex:	5 Males / 5 Females
Weight at dosing:	Males 241.6 – 257.4 g; Females 200.6 – 219.8g
Acclimation period:	5 days
Diet/Food:	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 67/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/ Switzerland) ad libitum
Water:	Tap water, ad libitum
Housing:	During acclimatization in groups of five per sex in Makrolon type-4 cages with standard softwood bedding.
Environmental conditions:	Temperature: 19 – 20 °C Humidity: 35 – 78% Air changes: 10 – 15/hour 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2006-12-14 to 2006-12-28

Animal assignment and treatment:

A dust aerosol was generated from the milled and pre-dried test item using a rotating brush aerosol generator (CR 3020, CR Équipements SA, CH-1295 Tannay, Switzerland) connected to a micronising jet mill. No extra diluent air was added. The generated aerosol was discharged into the exposure chamber through a ^{63}Ni charge neutraliser. The achieved mean aerosol concentration of 3.252 mg/L air administered for 4 hours was considered to represent the highest technically achievable concentration suitable for acute inhalation toxicity testing in rodents. An increase in aerosol concentration by an increased supply of test item to the rotating brush of the aerosol generator would have led to complete blockage of the rotating brush (which had happened in a pre-study technical trial not performed under GLP), and consequently to complete blockage of the aerosol generation and exposure system. Two generator cylinders containing test item were needed, in order to generate the highest technically achievable aerosol concentration over a 4-hour and 30-minute aerosol generation period.
The test atmosphere enters the top under slight positive pressure and is distributed to the entrance of each feed tube. It is then delivered through these tubes to the animal's nose. The inhalation exposure system is located inside a ducted extraction cabinet. Test atmosphere samples for the gravimetric measurements of the test item concentration and particle size distribution, and for the measurement of temperature, relative humidity and oxygen concentration, were collected directly from the feed tube in the breathing zone of the animals, at an

empty port of the exposure chamber delivering "fresh" test item to the animal's nose. This approach was chosen in order to obtain representative samples of what was delivered to the animals.
The particle size distribution was determined twice during the exposure using a Mercer 7 stage cascade impactor (Model 02-130, In-Tox Products Inc., Albuquerque, New Mexico, U.S.A.).

Representative samples of the test atmosphere were drawn through the impactor with a flow rate of 1.0 L/min and the particles deposited according to their aerodynamic size onto stainless steel slips and the final filter stage (Type HVLP, Polyvinylidenedifluoride membrane, pore size 0.45 µm), on each stage of the impactor. To obtain the mass deposited on each stage of the impactor, the steel slips and the final filter stage were carefully weighed before and after sampling using a Mettler MX5 analytical balance (Mettler AG, CH-8604 Volketswil, Switzerland). The total mass (µg) deposited in the impactor was then calculated by adding together the mass deposited on each of the stainless steel slips and the final filter stage. As the Effective Cut-off Diameters (ECD) represent the lower size limit of the particles collected on each stage, the cumulative percent less than the indicated size was tabulated as a function of the ECD (Aerosol Measurement, K. Willeke & P.A. Baron, editors, page 224, Van Nostrand Reinhold, New York
(1993)). This data was used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) using Microsoft Excel software. The target range for the MMAD was 1 to 4 µm, and was achieved.

Mortality was checked once daily during the acclimatisation period, once before exposure on the day of exposure (test day 1), at approximately 1, 2, 3 and 4 hours after exposure start, approximately 1 and 2 hours after the end of exposure on test day 1, and twice daily during the remainder of the observation period. Clinical signs were recorded at approximately 1, 2, 3 and 4 hours after exposure start and approximately 1 hour after the end of exposure on test day 1. In addition, clinical signs were recorded once daily on test days 2 to 15. Body weights were recorded on test days 1 (before exposure), 4, 8 and 15 (day of necropsy). At the end of the observation period, all animals were sacrificed. All animals were necropsied and were examined for any abnormalities.

II. RESULTS

A. TEST ATMOSPHERE

The nominal test aerosol concentration, the mean and standard deviation of the gravimetrically determined test aerosol concentration, and the Mass Median Aerodynamic Diameters (MMAD) and their Geometric Standard Deviations (GSD) are summarized in Table 5.2.3.9-1.

Table 6.2.3.8-6: Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] 2007): Details of test atmosphere

Nominal aerosol concentration (mg/L)	Mean achieved concentration (gravimetric) (mg/L) ± standard deviation	Mean Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
6.304	3.252 ± 0.053	2.95 (Impactor 1) 3.05 (Impactor 2)	2.97 (Impactor 1) 2.73 (Impactor 2)

The gravimetrically determined mean aerosol concentration was only 52% of the nominal aerosol concentration. This was attributed to accumulation of a large proportion of the test item in the aerosol generation and exposure system. The gravimetrically determined aerosol concentration can be regarded as stable over the 4-hour inhalation exposure period. This was evident from the low standard deviation.
The MMADs were within the range of 1 to 4 µm according to OECD 403 guideline. Therefore, satisfactory deposition of the particles can be assumed in the upper and the lower respiratory tract. Hence, the particle size distribution and MMADs obtained were considered to be appropriate for acute inhalation toxicity testing.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

The following clinical signs were recorded during and/or after the inhalation exposure, whereby the whole range of the stated severity grades was not necessarily recorded in each affected animal: Salivation, moderate in degree, and deep respiration in two male animals (nos. 3 & 5), and breath sounds [rales], slight to marked in degree, in three male (nos. 1, 3 & 5) and two female animals (nos. 6 & 10).
The findings of salivation and deep respiration were seen at approximately 3 and 4 hours after exposure start, when the animals were restrained in the exposure tubes. Deep respiration was still evident one hour afterwards, at approximately one hour after the end of the exposure period. Breath sounds [rales] were only noticed at approximately one hour after the end of the exposure period and on the day afterwards (test day 2) after the animals had returned to their housing cages. By two days after the inhalation exposure (test day 3) all clinical signs had cleared, and all animals remained free from clinical signs until the scheduled necropsy day (test day 15).
The clinical signs are summarized in the table below.

Table 6.2.3.8-7: Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] 2007): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day –Time after start of exposure (h)					Test Day				
			1	2	3	4	5	2	3	4	5	6-15
Deep respiration	Male	5	0	0	2	2	2	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0
Breath sound (rales)	Male	5	0	0	0	0	3 (marked or severe)	1 (moderate) 2 (marked or severe)	0	0	0	0
	Female	5	0	0	0	0	2 (moderate)	1 (slight) 1 (moderate)	1	0	0	0
Salivation	Male	5	0	0	2	2	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0

D. BODY WEIGHT

Losses in body weight were evident in three of five male animals (mean loss in the affected males –3.0 %) and three of five female animals (mean loss in the affected females –2.1 %), and retardation in body weight gain in one other male animal (+0.8 % weight gain) over the first three days following the inhalation exposure (test days 1 to 4). The effects on body weight were only transient and were followed by normal body weight gain in all animals. The body weight data are summarized in the table below.

Table 6.2.3.8-8: Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] 2007): Body weight data

Group		Day 1	Day 4	Day 8	Day 15
Male	Mean body weight (g)	252.1	249.2	276.8	312.1
	Standard deviation	7.2	12.1	12.4	13.0
Female	Mean body weight (g)	208.8	207.8	218.5	232.4
	Standard deviation	8.5	9.7	8.7	9.8

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical (NUP 05068) after a 4-hour exposure in male and female rats observed for a period of 15 days, was greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The highest technically achievable concentration of 3.252 mg/L air was tested. The MMAD was within the recommended range according to the guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC50 is >3.252 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the acute inhalation LC_{50} >3.252 mg/L air in male and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.3.9. Study 9

Data point:	CA 5.2.3/009
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats – Limit Test
Report No	15276
Document No	Not reported
Guidelines followed in study	OPPTS 870.1300 (1998), OECD 403 and JMAFF 59 NohSan No. 4200 (1985).
Deviations from current test guideline (OECD 403, 2009)	There were no deviations from the Study Plan. Limit test performed on 5 animals per sex. Body weight measured at Day 0

	and once a week afterwards. The humidity and number of air changes was not specified. The GSD is not reported. The tested concentration is considered low for a limit test and the study report does not describe attempts to increase the concentration tested while maintaining a respirable size distribution. Therefore, the study is considered acceptable, but with restrictions
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 (reliable with restrictions)

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined gravimetrically, was 2.04 mg/L air for 4 hours. The analysis of the particle size distribution was carried out by a cascade impactor and the mass median aerodynamic diameter (MMAD) was estimated to be 2.5 µm. All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be >2.04 mg/L air (actual concentration).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate Acid Technical

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Stability of test compound:	Test substance was expected to be stable for the duration of testing.

2. **Test animals:**

Species:	Rat albino
Strain / Stock:	Sprague-Dawley derived
Source:	[redacted]
Age:	9-10 weeks
Sex:	5 male and 5 female
Weight at dosing:	Males: 280 – 318 g; Females: 205 – 224 g
Acclimation period:	13 days
Diet/Food:	Purina Rodent Chow #5012, *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.

Environmental conditions: Temperature: 19 – 23 °C
12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2003-05-17 to 2003-05-24

Animal assignment and treatment:

The study was carried out using a nose-only inhalation chamber with an internal volume of approximately 6.7 L and approximately 283 air changes per hour during the study. Animals were individually housed in polycarbonate holding tubes. The test item was micronized before administration and aerosolized using a dust generator which was directly connected to the inhalation chamber. Gravimetric samples were withdrawn at 6 intervals from the breathing zone of the animals to gravimetrically determine the dust concentration in the inhalation chamber. Particle size distribution of the test atmosphere was determined with an Andersen Cascade Impactor. Samples were withdrawn from the breathing zone of the animals at two intervals. Animals were exposed for 4 hours and 1 minute to an actual concentration of 2.04 mg/L air (determined gravimetrically).
Observations for mortality and clinical/behavioural signs of toxicity were made upon removal from the exposure chamber and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to test substance exposure and on Days 7 and 14.
On Day 14 after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS

A. DUST CONCENTRATION AND PARTICLE SIZE DISTRIBUTION

The gravimetric and nominal chamber concentrations were 2.04 and 8.99 mg/L, respectively. The mass median aerodynamic diameter was estimated to be 2.5 µm based on the particle size distribution as measured with an Andersen Cascade Impactor.

B. MORTALITY

There were no mortalities during the study.

C. CLINICAL OBSERVATIONS

All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period.

D. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rat, observed a period of 14 days was greater than 2.04 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only

minor deviations to the current valide OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is > 2.04 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered acceptable, but with restrictions. The tested concentration is considered low for a limit test and the study report does not describe attempts to increase the concentration tested while maintaining a respirable size distribution. Under the conditions of the study the acute inhalation LC_{50} > 2.04 mg/L air in male and female rats.

B.6.2.3.10. Study 10

Data point:	CA 5.2.3/010
Report author	[redacted]
Report year	2004
Report title	An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623
Report No	3044.969
Document No	[redacted]2003-116
Guidelines followed in study	EC method B.2, OECD 403 (1981), EPA Health Effects Test Guidelines, OPPTS 870.1300, JMAFF 12 Nohsan No. 8147
Deviations from current test guideline (OECD 403, 2019)	Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards.
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

The four-hour nose-only inhalation toxicity of the test substance MON 78623 containing 47.2 % glyphosate (57.8 % potassium salt of glyphosate) was evaluated in Sprague Dawley rats. Two limit tests were performed in which one group of five male and five female rats each received a four-hour nose-only inhalation exposure to a time-weighted average aerosol concentration (analytically determined) of 2.21 or 5.27 mg/L. For the first exposure (2.21 mg/L) the mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.9 µm and 2.18, respectively. The percentage of particles ≤ 4.0 µm was determined to be 67 %. Since there was no mortality, a second limit test was conducted at a greater target concentration. For the second exposure (5.27 mg/L) the mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 3.8 µm and 2.20, respectively. The percentage of particles ≤ 4.0 µm was determined to be 53 %. Following each exposure, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).
No mortality occurred for the 2.21 mg/L dose level. The most notable clinical abnormalities observed during the study included transient incidences of congested breathing and dark material around the facial area. Body weight gain was noted for all animals during the test period. No gross internal findings were observed at necropsy on study day 14.

No mortality occurred for the 5.27 mg/L dose level. The most notable clinical abnormalities observed during the study included transient incidences of congested breathing and few feces. Slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals during the test period and all animals exceeded their initial body weight at study termination. No gross internal findings were observed at necropsy.

The acute inhalation LC_{50} was calculated to be >5.27 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	MON 78623
Description:	Clear colourless liquid (pipet), light amber liquid (bulk)
Lot/Batch #:	GLP-0306-14124-F
Purity:	47.2 % glyphosate (57.8 % potassium salt of glyphosate)
Stability of test compound:	Expiry June, 2004

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	Sprague Dawley
Source:	[redacted]
Age:	8-9 weeks
Sex:	Males and females
Weight at dosing:	Males: 276 – 312 g; females: 182 – 210 g
Acclimation period:	At least 5 days
Diet/Food:	PMI Certified Rodent Chow #5002 (PMI Nutrition International), *ad libitum* (except during acclimatization to the exposure tubes and during the exposure)
Water:	Tap water, *ad libitum* (except during acclimatization to the exposure tubes and during the exposure)
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 19 – 23 °C
	Humidity: 31 – 65 %
	Air changes: 10-15 per hour
	Light cycle: 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2003-10-29 to 2003-12-29

Test atmosphere generation:

The test aerosol was generated with a Master Flex Pump and Pump Head (7523-30 and 77200-60) and a Pistol Spraying System. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5 L Elutriator, the Multi-Stage 10 L nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower.

Exposure chamber conditions:

Air flow readings were recorded at the initiation of the T99 equilibration period, at approximately 30-minute intervals during each aerosol exposure and at the conclusion of the de-equilibration period. The aerosol concentration was measured at the beginning of each aerosol exposure (after equilibration), at approximate 30-

minute intervals during the aerosol exposure, and at the conclusion of each aerosol exposure (before de-equilibration). Samples of the test article aerosol were collected in the inhalation chamber by gravimetric technique. Both gravimetric and analytical aerosol concentrations were determined. A 5 L sample of the aerosol was drawn from the breathing zone of the animals in the chamber through a pre-weighed glass fiber filter. For the analytical concentration, the gravimetrically obtained samples were analyzed by liquid chromatography for the non-volatile glyphosate component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. Chamber oxygen content was measured and recorded at approximate 30-minute intervals during each aerosol exposure using a GC-501 Oxygen Detector.

Particle size distribution:

The aerosol aerodynamic particle-size distribution was determined three times during each aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a pre-weighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently determined using an Excel computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles ≤ 4.0 µm were then determined. The results were as follows:

Table 6.2.3.10-9: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 (2004): Details of Test Atmosphere

Mean Achieved Actual Concentration (analytical method)	MMAD	GSD	Respirable Amount Particle Size ≤ 4 µm
(mg/L)	(µm)		(%)
2.21	2.9	2.18	67
5.27	3.8	2.20	53

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

Animal assignment and treatment:

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least one hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable. Animals that did not appear to acclimate to the exposure tube were not acceptable. All animals were removed from the exposure tubes and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multi-Stage 10 L nose-only inhalation chamber and the test article aerosolized at the following levels:

6.2.3.10-10: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 2004): Dose Levels

Analytical Exposure Level (mg/L)	No. of Animals	
	Male	Female
2.21	5	5
5.27	5	5

Each aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces

(where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water.

The limit test animals were observed for clinical abnormalities during each aerosol exposure (no positive clinical observations were noted during either exposure), two times on study day 0 (post-exposure) and daily thereafter (days 1-14). Individual body weights were recorded just prior to dosing and weekly thereafter. On Day 14 after dosing, each animal was euthanized and all study animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

The most notable clinical abnormalities observed for the 2.21 mg/L dose level included transient incidences of congested breathing and dark material around the facial area.

The most notable clinical abnormalities observed for the 5.27 mg/L dose level included transient incidences of congested breathing and few faeces.

The clinical signs are summarized in the table below.

Table 6.2.3.10-11: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 [redacted] 2004): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Day of study								
			0	1	2	3	4	5	6	7	8-14
Dose level: 2.21 mg/L											
Congested breathing	Male	5	3	3	0	0	0	0	0	0	0
	Female	5	4	1	0	0	0	0	0	0	0
Dark material around eye(s)	Male	5	1	0	0	1	0	0	0	0	0
	Female	5	3	0	0	0	0	0	0	1	0
Dark material around nose	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	1	0	0	0	0	0	0	0	0
Few faeces	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0
Dose level: 5.27 mg/L											
Congested breathing	Male	5	4	1	1	0	0	0	0	0	0
	Female	5	5	0	0	0	0	0	0	0	0
Dark material around eye(s)	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0
Dark material around nose	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0
Few faeces	Male	5	0	0	0	0	0	0	0	0	0
	Female	5	0	2	0	0	0	0	0	0	0

C. BODY WEIGHT

Body weight gain was noted for all animals for the 2.21 mg/L dose level.

For the 5.27 mg/L dose level, slight body weight loss was noted for two females during the day 0 to 7 body weight interval and for one female during the day 7 to 14 body weight interval. Body weight gain was noted for all other animals and all animals exceeded their initial body weight at study termination.
The body weight data are summarized in the table below.

Table 6.2.3.10-12: An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 [redacted] 2004): Body weight data

Dose level: 2.21 mg/L		**Day 0**	**Day 7**	**Day 14**
Male	Mean body weight (g)	298	313	344
	Standard deviation	10.0	17.5	15.6
Female	Mean body weight (g)	187	201	217
	Standard deviation	4.7	7.3	9.5
Dose level: 5.27 mg/L		**Day 0**	**Day 7**	**Day 14**
Male	Mean body weight (g)	284	311	331
	Standard deviation	8.4	11.2	14.9
Female	Mean body weight (g)	201	207	213
	Standard deviation	8.7	14.4	8.1

D. NECROPSY

No gross internal findings were observed at necropsy for the 2.21 mg/L and 5.27 mg/L dose levels on study day 14.

III. CONCLUSIONS

The acute inhalation LC_{50} of the test substance MON 78623 containing 47.2 % glyphosate (57.8 % potassium salt of glyphosate) after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.27 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current OECD 403 guideline (2009) and according to GLP. The test substance contained 47.2 % glyphosate. The MMAD and GSD were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >5.27 mg/L air. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the acute inhalation LC_{50} >5.27 mg/L air in male and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.3.11. Study 11

Data point:	CA 5.2.3/011

Report author	[redacted]
Report year	1999
Report title	NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats – Limit test
Report No	7909
Document No	Not reported
Guidelines followed in study	Health Effects Test Guidelines, OPPTS 870.1300 (1998)
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. Instead of nose-only exposure the animals were placed in whole body plexiglass chambers for the experiment, which is considered worst-case. The tested concentration is considered low for a limit test.
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

After establishing the desired generation procedures during pre-test trials, ten healthy rats were exposed to isopropylamine glyphosate (NUP5a99 (62 % glyphosate MUP)) 2.08 mg/L for 4 hours. Chamber concentration and particle size distributions of the test substance were determined periodically during the exposure period. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Body weights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice. All animals survived exposure to the test atmosphere and gained bodyweight over the 14-day observation period. The gravimetric chamber concentration was 2.08 mg/L. Based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor, the mass median aerodynamic diameter was estimated to be 2.6 microns. In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study. Gross necropsy findings at terminal sacrifice were unremarkable. Based on the results of this study, the single exposure

acute inhalation LC_{50} of NUP5a99 62 % glyphosate MUP is >2.08 mg/L air, the maximum achievable concentration.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	NUP5a99 62 % glyphosate MUP
Description:	clear viscous amber liquid
Lot/Batch #:	Drum Sample E
Purity:	62%
Stability of test compound:	No data available

2. **Vehicle and/ or positive control:** None
3. **Test animals:**

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	[redacted]
Age:	Not specified
Sex:	5 males and 5 females
Weight at dosing:	Males: 224 – 256 g; females: 179 – 201 g
Acclimation period:	10 days
Diet/Food:	Purina Rodent Chow #5012
Water:	Tap water, *ad libitum*
Housing:	individually housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 22 – 24 °C Humidity: not specified Air changes: not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: August 6-20, 1999

Test atmosphere generation:

Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures for achieving as closely as possible the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter ≤ 4 /µm). The test atmosphere was generated using 0.25 inch JCO atomizer, FC4 fluid cap and AC1502 air cap (Spraying Systems Inc.). Compressed air was supplied at 30 psi. The test substance was metered to the atomization nozzle through Size 14 Master Flex Tygon tubing, using a Master Flex Pump Model 7520-35.

Exposure chamber conditions:

The animals were placed in a rectangular whole body plexiglass chamber with a volume of 150 liters with prechamber operated under slight negative pressure, and were exposed to the test atmosphere for 4 hours and 15 minutes. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T_{99}).

Particle size distribution:

An eight-stage Andersen cascade impactor was used to assess the particle size distribution of the test atmosphere. Samples were withdrawn from the breathing zone of the animals at two intervals. The aerodynamic mass median diameter and geometric standard deviation were determined graphically using two-cycle logarithmic probit axes. The mass median aerodynamic diameter was estimated to be 2.6 µm based on the particle size distribution.

Table 6.2.3.11-13: NUP5a99 62 % glyphosate MUP: Acute inhalation toxicity study in rats – Limit test ([redacted] 1999): Details of test atmosphere

Chamber concentration (mean)*	Standard deviation	MMAD (mean of 2 samples)	GSD (mean of 2 samples)
(mg/L air)		(µm)	
2.08	0.07	2.6	1.72

* The chamber concentration was measured every 30 minutes.
MMAD = mean mass median aerodynamic diameter

GSD = geometric standard deviation

Animal assignment and treatment:

A group of five rats per sex received the test material at a dose level of 2.08 mg/L by whole body exposure. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

II. RESULTS

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

In-chamber animal observations included ocular and nasal discharge, hunched posture and hypoactivity. Apart from test substance noted on the fur, all animals recovered from the above symptoms upon removal from the exposure chamber and appeared active and healthy throughout the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSIONS

The acute inhalation LC_{50} of isopropylamine glyphosate (NUP5a99 62 % glyphosate MUP) after a 4-hour exposure to male and female rats, observed over a period of 14 days is greater than 2.08 mg/L air.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline and according to GLP. The test substance isopropylamine glyphosate contained 62 % glyphosate MUP. The MMAD and GSD were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} of isopropylamine glyphosate is >2.08 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered acceptable. Under the conditions of the study the LC_{50} > 2.08 mg/L air in male and female rats (highest tested concentration).

B.6.2.3.12. Study 12

Data point:	CA 5.2.3/012
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats

Report No	[redacted] P/4882
Document No	Not reported
Guidelines followed in study	OECD 403 (1981): OPPTS 870.1300 (1998): 92/69/EEC B.2 (1992) + amendment 93/21/EEC (1993)
Deviations from current test guideline (OECD 403, 2009)	Bodyweights were recorded on Day -1, 8 and prior to termination on day 15. According to the current guideline, animal weights should be recorded once during the acclimatization period, on the day of exposure prior to exposure (day 0), and at least on days 1, 3 and 7 (and weekly thereafter), and at the time of death or euthanasia if exceeding day 1. During the exposure period the temperature and humidity of the room were outside the specified range. Time required to reach inhalation chamber equilibrium is not reported. These deviations are not considered to impact the outcome of the study.
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

In an acute inhalation toxicity study, groups of five male and five female Alpk:APfSD (Wistar-derived) rats were exposed nose-only for a single four-hour period to glyphosate technical at target particulate concentrations of 5 mg/L and 2 mg/L. The particle size distribution of the test atmosphere was analysed at frequent intervals during the exposure period. Following exposure, the animals were retained without treatment for 14 days. Clinical observations and bodyweights were recorded and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem.*

The achieved test atmosphere had the following characteristics:

Table 6.2.3.12-14: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] 1996): Details of test atmosphere

Target concentration mg/L	Achieved particulate concentration mg/L	MMAD* µm	GSD+
2	2.47± 0.15	3.57, 3.03	1.94, 1.90
5	4.43 ± 1.297	2.91, 3.41	1.74, 2.04

* Mass Median Aerodynamic Diameter (µm)
+ Geometric Standard Deviation

Two males and two females exposed to 4.43 mg glyphosate technical/L were found dead or were terminated *in extremis* during the observation period, the remaining animals in this group survived until scheduled termination. Clinical signs indicative of moderate toxicity was seen in this group. All surviving animals had regained their initial bodyweight by the end of the study.

Similar but less severe clinical signs were seen in animals exposed to 2.47 mg/L, all animals survived and showed complete recovery by the end of the study. All animals exposed to 2.47 mg/L survived to scheduled termination.

It was concluded that the acute inhalation LC_{50} of glyphosate technical exceeded 4.43 mg/L air for male and female rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate acid
Description:	Technical; white solid
Lot/Batch #:	P25
Purity:	95.6 % w/w
Stability of test compound:	Confirmed by Sponsor

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	Alpk:APfSD
Source:	[redacted]
Age:	Young adult; 9 – 12 weeks old at delivery
Sex:	Males and females
Weight at dosing:	243 – 365 g (males); 210 – 247 g (females) at the start of exposure
Acclimation period:	At least five days
Diet/Food:	PCD diet (Special Diet Services Limited, Witham, Essex, UK), *ad libitum* except during exposure.
Water:	Mains water, *ad libitum* except during exposure
Housing:	5 per cage, sexes separately, except during exposure, in rat racks suitable for animals of the strain and weight range expected during the study
Environmental conditions:	Temperature: 21 ± 2 °C
	Humidity: 40 – 70 %
	Air changes: at least 15/hour
	Photoperiod: 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-11-22 to 1996-03-11

Exposure conditions: Trial generations were carried out prior to the start of the study in order to determine the appropriate generation system and conditions, to determine the appropriate target concentration that could be achieved, or if not, what was the maximum stable attainable concentration, to obtain data on the aerodynamic particle size of the atmosphere generated, to determine an appropriate method of analysis of glyphosate acid. Exposure conditions during the study are given later in a table of the test atmosphere characteristics of glyphosate acid.

Animal assignment and treatment: The study consisted of two main study groups of 5 rats/sex/group exposed nose-only for a single four-hour period to glyphosate acid at target particulate concentrations of 5 mg/L and 2 mg/L. A third group (unassigned) of 2 males and 2 females was used for trial exposures to the target concentration. Prior to the start of the study the rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure they were observed frequently and, at the end of the 4-hour exposure period, each rat was given a detailed clinical examination. They were also subjected to detailed clinical observations, daily during a 14-day observation period. The bodyweight of each rat was recorded on day -1, 8

and prior to termination on day 15. All rats were killed on day 15 and subjected to a gross examination *post mortem* involving external observation and careful internal examination of all thoracic and abdominal viscera.

Generation of the test atmosphere / chamber description: Before exposure of the test animals, the atmosphere was shown to have been acceptably stable. The test atmosphere was generated using a modified Wright's dust-feed mechanism. Clean, dry air was passed through the dust feed at a nominal flow rate of 2.5 L/minute (at normal temperature and pressure) and carried the atmosphere to the exposure chamber, having an internal volume of 27.6 litres. Since diluting air was not employed, the flow rate through the exposure chamber was the same as that employed in the generation of the test atmosphere. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flow-meters and were altered as necessary to maintain target concentration. Animals were exposed nose-only to the atmosphere. They were restrained in polycarbonate tubes (Battelle, Switzerland), which were inserted into the Perspex exposure chamber. The chamber was covered with an aluminium cone and stood on an aluminium base.

Test atmosphere concentration: The particulate concentration of the test atmosphere, close to the animals' breathing zone, was measured gravimetrically at frequent intervals during the exposure period. This was done by drawing the test atmosphere, at a known flow rate, for a known time, through a 25 mm diameter, polyvinyl chloride (PVC) GLA 5000 filter housed in a Delrin open-faced filter holder. The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{Concentration (mg/L)} = \frac{\text{post wt (mg)} - \text{pre wt (mg)}}{\text{time (minutes) x airflow (L/minute)}}$$

Pre wt = weight of filter prior to sampling
Post wt = weight of filter after sampling

Particle size determination: The aerodynamic particle size distribution of the test atmosphere was measured twice during the exposure period, using a Marple Cascade Impactor, which aerodynamically separates airborne particles into pre-determined size ranges. Using a microcomputer, the data were transformed using a log/probit transform and a linear regression derived from the cumulative data. The linear regression line was then used to calculate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

Table 6.2.3.12-15: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] 1996): Summary of acute study test atmosphere characteristics

Parameter	Target concentration 5 mg/L				Target concentration 2 mg/L			
Measured particulate concentration	4.43 ± 1.30 mg/L				2.47 ± 0.15 mg/L			
% total particulate	96.9 ± 4.2				98.5 ± 7.7			
Analysed concentration of glyphosate acid(mg/L)	Mean 4.27 ± 1.15				Mean 2.43 ± 0.19			
Particle size MMAD; GSD	2.91, 3.41µm; 1.74, 2.04				3.57, 3.03µm; 1.94, 1.90			
Size range (µm)	% by weight in range				% by weight in range			
	Run 1 (1hr 35min into exposure)		Run 2 (3hr 29min into exposure)		Run 1 (54min into exposure)		Run 2 (2hr 59min into exposure)	
	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Grav-imetric
Particles > 9.8 µm (% w/w)	0.9	0.7	5.1	4.3	3.1	3.1	2.0	2.0
Particles 9.8-6.0 µm (% w/w)	21.1	20.6	26.3	23.8	20.9	23.0	16.7	16.4
Particles 6.0-3.5 µm (% w/w)	34.4	35.5	34.3	31.3	47.9	46.4	37.7	36.8
Particles 3.5-1.55 µm (% w/w)	32.1	32.3	21.3	28.0	19.5	18.8	38.0	36.9
Particles 1.55-0.93 µm (% w/w)	7.5	7.6	9.1	8.6	5.9	5.4	3.6	4.0
Particles 0.93-0.52 µm (% w/w)	2.2	2.3	2.7	2.3	1.9	2.0	1.3	1.6
Particles ≤0.52 µm (% w/w)	1.5	1.0	1.3	1.8	0.8	1.3	0.8	2.4
Flow rate (whole system)	2.5 L/min							
Temperature	14.7 – 21.7 °C							
Humidity	25 – 65 %							

- Percentages are calculated as follows:
Gravimetric: $\frac{\text{weight trapped at each size range x 100}}{\text{Total weight trapped}}$

Statistics: The acute inhalation LC_{50} was estimated.

II. RESULTS

A. MORTALITY

Two males and two females exposed to 4.43 mg/L air were found dead or were terminated in extremis on days 5, 6 or 9 of the study, the remaining animals in this group survived until scheduled termination.

There were no mortalities at 2.47 mg/L air.

Table 6.2.3.12-16: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] 1996): Mortality / animals treated

Target exposure		Cumulative mortality (Number dead / total)		
concentration mg/L	Day number	Males	Females	Combined
5	5	1/5	0/5	1/10
	6	2/5	1/5	3/10
	9	2/5	2/5	4/10
	14	2/5	2/5	4/10
2	14	0/5	0/5	0/10

B. CLINICAL OBSERVATIONS

Abnormalities generally associated with restraint (wet fur) were seen in all animals during exposure. Clinical changes seen were salivation, irregular breathing and auditory hypoaesthesia, these effects were considered to be related to treatment.

Immediately after exposure, abnormalities generally associated with restraint (hunched posture, piloerection and wet fur) were seen in both males and females. At an exposure concentration of 4.43 mg/L the clinical abnormalities seen in both sexes included breathing irregularities, reduced righting reflex, shaking, splayed gait and were considered to be indicative of moderate toxicity.

At an exposure concentration of 2.47 mg/L the number of adverse clinical changes observed was reduced in both sexes. Those abnormalities observed were similar to those seen in animals exposed to 4.43 mg/L glyphosate acid.

The clinical condition of most animals appeared to have improved by day 5 of the study, with the exception of 2 males and 2 females exposed to 4.43 mg/L. There was generally an improvement in clinical condition during the remainder of the study.

Table 6.2.3.12-17: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] 1996): Clinical observation data during exposure

Group	Time into exposure (min)	Abnormalities*
4.43 mg/L	30	Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	60	All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	90	All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	120	All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	150	Some animals: lachrymation All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	180	Some animals: lachrymation

		All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
	210	Some animals: lachrymation All animals: salivation Most animals: wet fur, changes in breathing rate and depth and reduced response to sound
2.47 mg/L	30	Some animals: wet fur, changes in breathing rate and depth
	60	Some animals: wet fur, changes in breathing rate and depth
	90	Most animals: wet fur, reduced response to sound Some animals: changes in breathing rate and depth
	120	Most animals: wet fur, reduced response to sound Some animals: changes in breathing rate and depth
	150	All animals: wet fur, no response to sound, reduced breathing rate and increased breathing depth or irregular breathing, test substance staining nose Some animals: gasping, lachrymation
	180	All animals: wet fur, no response to sound, reduced breathing rate and increased breathing depth or irregular breathing, test substance staining nose Some animals: gasping, lachrymation
	210	All animals: wet fur, no response to sound, reduced breathing rate and increased breathing depth or irregular breathing, test substance staining nose Some animals: gasping, lachrymation

* No individual animal data provided in the study report.

e 6.2.3.12-18: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats 1996): Clinical observation data following exposure

Clinical sign	**Sex**	**No. of animals exposed**	**Day following exposure**														
Group 1: 4.43 mg/L			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Activity decreased	Male	5	5	1													
	Female	5	5					1									
Breathing depth	Male	5	2														
	Female	5	1								1						
Breathing rate	Male	5	2														
	Female	5	1								1						
Breathing irregular	Male	5	5	5	3	3		1									
	Female	5	4	5	1												
Gasping	Male	5	3														
	Female	5	2					1									
Hunched	Male	5	5	5	5	5		3	3	3	3	3	3	3			
	Female	5	5	5	5	5		5	4	4	4	3	3	3	2		
Found dead (M) / Killed in extremis-toxic (F)	Male	5					1	1									
	Female	5						1			1						
Killed termination	Male	5															3
	Female	5															3
Piloerection	Male	5	5	5	5	5		3	3								
	Female	5	5	5	5	5		5	4								

Abnormal respiratory noise	Male	5	5	5	5	5	4	3	3								
	Female	5	5	4	2	2	5	2	1	1	1						
Reduced righting reflex	Male	5	1														
	Female	5															
Salivation	Male	5	5														
	Female	5	5														
Shaking	Male	5	5														
	Female	5	5														
Splayed gait	Male	5	5	5	5	5		3									
	Female	5	1	1	1												
Sides pinched in	Male	5															
	Female	5							1	1	1						
Reduced stability	Male	5	3														
	Female	5	5														
Signs of urinary incontinence	Male	5		4													
	Female	5		2													
Tail erection	Male	5															
	Female	5	1	1	1	1		1	1								
Thin	Male	5															
	Female	5						1									
Test substance round snout	Male	5	5														
	Female	5	5														
Vocalisation	Male	5					4										
	Female	5					5										
Wet fur	Male	5	5														
	Female	5	5														
Group 2: 2.47 mg/L																	
Activity decreased	Male	5	1														
	Female	5															
Breathing depth	Male	5	4	4	3	1											
	Female	5	2	2													
Breathing rate	Male	5	4	4	2												
	Female	5	3	3													
Breathing irregular	Male	5	2	2	1	2											
	Female	5	2	2	2	2											
Chromodacryorrhea	Male	5	1														
	Female	5															
Head flicking	Male		1														
	Female																
Hunched	Male	5	5	5	5	5	1										
	Female	5	5	5	5	5	1										
Killed termination	Male	5															5
	Female	5															5
Lachrymation	Male		2														
	Female																
Paw flicking	Male		1														
	Female																
Piloerection	Male	5	5	5	5	5	5	3									
	Female	5	5	5	5	5	5	4									
Abnormal respiratory noise	Male	5	5	5	4	3	3	3	2	2	1	1	1	1	1	1	1
	Female	5	4	4	4	2	1	1									

Salivation	Male	5	5														
	Female	5	5														
Scabs	Male	5												1	1	1	1
	Female	5															
Shaking	Male	5	2														
	Female	5															
Reduced stability	Male	5	1														
	Female	5	1														
Test substance round snout	Male	5	5														
	Female	5	5	1													
Wet fur	Male	5	5														
	Female	5	5														

C. BODYWEIGHT

Animals showed a treatment related reduction in bodyweight. At an exposure concentration of 4.43 mg/L all animals had exceeded their initial bodyweight by the end of the study. At an exposure concentration of 2.47 mg/L all animals had exceeded their initial weight by day 8 of the study.

The body weight data are summarized in the table below.

le 6.2.3.12-19: Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats 1996): Body weight data

Group 1: 4.43 mg/L		**Day 1**	**Day 2**	**Day 3**	**Day 8**	**Day 15**
Male	Mean body weight (kg)	350.6	313.8	306.0	342.7	374.7
	Standard deviation	13.2	14.6	29.2	20.5	18.2
Female	Mean body weight (kg)	227.4	208.0	210.0	210.8	236.3
	Standard deviation	14.0	18.7	15.2	31.5	19.6
Group 2: 2.47 mg/L		**Day 1**	**Day 2**	**Day 3**	**Day 8**	**Day 15**
Male	Mean body weight (kg)	252.8	234.2	246.6	281.2	323.0
	Standard deviation	9.8	11.0	11.4	14.2	18.8
Female	Mean body weight (kg)	216.8	203.0	212.6	228.8	237.0
	Standard deviation	3.9	8.0	10.0	8.2	7.0

D. NECROPSY

In the animals exposed to 4.43 mg/L that died or were killed prior to termination, the two males found dead had dark lungs (probably a result of agonal congestion), the lungs of the females were normal.

At scheduled termination, the lungs of rats exposed to 4.43 mg/L were normal. One female exposed to 2.47 mg/L had red spots on the lungs and another female had dark lungs. These findings are considered to be

incidental to treatment. Changes at necropsy in a variety of tissues in males exposed to 2.47 mg/L were of low incidence and were considered to be unrelated to treatment.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 4.43 mg/L air.

Assessment and conclusion

Assessment and conclusion by applicant:
The study is performed in accordance with the current OECD 403 guideline (2009) and according to GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Therefore, the study is acceptable. Based on the study results, the acute inhalation LC_{50} is > 4.43 mg/L air for male and female rats. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the acute inhalation LC_{50} > 4.43 mg/L air in male and female rats. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.3.13. Study 13

Data point:	CA 5.2.3/013
Report author	[redacted]
Report year	1995
Report title	HR-001: Acute inhalation toxicity study in rats
Report No	[redacted]94-0155
Document No	Not reported
Guidelines followed in study	U.S. EPA FIFRA Guideline Subdivision F, OECD 403 (1981)
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD (4.8 ± 0.3 µm with a GSD of 1.7 ± 0.1). Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week thereafter. Instead of nose-only exposure the animals were placed in whole body exposure chambers for the experiment, which is considered worst-case. Based on the high MMAD the study is considered reliable with restrictions. ***In the study report two different lot numbers and purities are reported for the test substance. As no certificate of analysis was attached to the study report, the applicant is asked to further clarify which lot and purity has been used for the test.***
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the MMAD exceeding the recommended range

Five Fischer (F344/DuCrj) rats of each sex were exposed to glyphosate technical mist for 4 hours in a whole body exposure chamber followed by clinical observations for 14 days. Body weight of each animal was measured prior to exposure (day 0) and on days 7 and 14.

After the end of the observation period, all animals were euthanized and necropsied.

Mean actual atmospheric concentration of HR-001 was 5.48 mg/L. Mean values of mass median aerodynamic diameters and geometric standard deviations were 4.8 ± 0.3 and 1.7 ± 0.1 µm. More than 91 % of the test substance consisted of particles with an aerodynamic diameter less than 10 µm or less. Clinical observations of animals during the exposure was impossible due to heavy foggy test substance mist in the chamber. There were no deaths in either sex during exposure or the subsequent 14-day observation period. After the termination of exposure, wetted fur in the perioral and in periocular regions, and red adhesive materials in the periocular and in nasorostral regions were observed. These signs were respectively slight in degree and disappeared by day 4 in males and day 5 in females. All animals gained the expected body weight on day 7 and 14 when compared with those on day 0. No abnormalities were detected in any animal of either sex at final necropsy after the end of observation period.

The acute inhalation LC_{50} was calculated to be >5.48 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate TC
Description:	Whitish crystals
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not mentioned in the report.

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	F344/DuCrj
Source:	[redacted]
Age:	8 weeks
Sex:	Males and females
Weight at dosing:	♂ 176 – 187 g; ♀ 138 – 144 g
Acclimation period:	8 days
Diet/Food:	certified pellet diet MF (Lot No. 950109, Oriental Yeast Co., Ltd., Tokyo), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	By group of 5 animals of the same sex in stainless steel wire cages during pre- and post-exposure periods. Individually in stainless steel wire cages during exposure.
Environmental conditions:	Temperature: 22 ± 2 °C Humidity: 55 ± 15 % Air changes: 10/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1995-03-28 to 1995-04-20

Test atmosphere generation:

The dust was generated by a turn-table type dust feeder with compressed air from air compressor. The compressed air was supplied to the dust feeder through an air filter. The air was introduced into the chamber as diluting air after filtering it through a HEPA filter.

Exposure chamber conditions:

The nominal atmospheric concentration of HR-001 was calculating by dividing the total amount of the test substance supplied to the dust feeder during the 4-hour exposure by the total air volume delivered during the exposure.

The actual atmospheric concentration was measured gravimetrically and analysed by high-performance liquid chromatography (HPLC).

Particle size distribution:

The results for the air samples taken for the determination of particle size distribution are given in the table below.

Table 6.2.3.13-20: HR-001: Acute inhalation toxicity study in rats [redacted] 1995): Particule size distribution

Exposure group	Time of sampling	Analytical concentration	Particule size	
mg/L	(min)	(mg/L)	MMAD[1] (µm)	σg[2]
5.48	60	6.54	5.0	1.6
	120	4.80		
	180	5.11	4.6	1.8
	Mean	5.48	4.8	1.7
	S.D.[3]	0.93	0.3	0.1

[1] MMAD: Mass median aerodynamic diameter
[2] σg: geometric standard deviation
[3] S.D.: Standard deviation

The results revealed a MMAD of 4.8 µm (σg = 1.7) during the exposure period. Thus more than 91 % of the test substance dust consisted of particles of inhalable size.

Animal assignment and treatment:

Groups of 5 male and 5 female specific pathogen free Fisher rats (F344/DuCrj) were exposed (whole-body) continuously for 4 hours to test substance mist containing concentrations of glyphosate at 5.48 mg/L. The flow rate was stable at approximately 100 L/min (16 air changes/h). Mortality and signs of reaction to treatment were recorded during a subsequent 14-day observation period. All animals were observed for clinical signs at 2 hours after the initiation of exposure, immediately and at 2 hours after the termination of exposure. In addition, animals were observed for lethality at 4 hours after the termination of exposure. All animals were weighed shortly before the exposure and on days 7 and 14. The surviving animals were euthanized on the following day (day 15). All animals were subjected to necropsy.

II. RESULTS

A. MORTALITY

There were no deaths in either sex at the tested concentration of 5.48 mg/L.

B. CLINICAL OBSERVATIONS

No clinical observations were performed during the exposure due to foggy dust in the exposure chamber. No notable serious changes were observed as clinical signs after the exposure period. Wetted and soiled fur in the periocular and nasorostral regions were not considered to be particularly caused by the test substance because the changes were slight in degree and are frequently observed in the acute inhalation toxicity study. The clinical signs are summarized in the table below.

Table 6.2.3.13-21: HR-001: Acute inhalation toxicity study in rats [redacted] 1995): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Exposure Day (h)			Post-exposure Day				
			During exposure[1]	After exposure						
			2	0	2	1	2	3	4	5-14
Wetted fur in perioral region	Male	5	-	5	0	0	0	0	0	0
	Female	5	-	5	0	0	0	0	0	0
Red adhesive materials in periocular region	Male	5	-	5	4	4	3	1	0	0
	Female	5	-	1	2	5	1	0	0	0
Wetted fur in periocular region	Male	5	-	5	0	0	0	0	0	0
	Female	5	-	5	0	0	0	0	0	0
Red adhesive materials in nasorostral region	Male	5	-	0	4	4	2	1	0	0
	Female	5	-	0	5	2	1	1	1	0

[1]Animals were not observed due to foggy dust in the chamber

C. BODY WEIGHT

All animals gained weights, reflecting their good healthy conditions.

D. NECROPSY

No abnormalities were observed in any animal of either sex at necropsy.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was greater than 5.48 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD slightly exceeded the recommended value of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is > 5 mg/L air after an exposure period of 4 hours. According to the classification criteria of the CLP Regulation (EU) No. 1272/2008, no classification is required.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered acceptable but with restrictions (reliable with restrictions) as the MMAD exceeds the range recommended in the OECD guidance. The animals were exposure in a whole body exposure chamber instead of nose-only and therefore other routes of exposure cannot be excluded, which is considered worst-case. Under the conditions of the study the LC_{50} > 5 mg/L air in male and female rats.

B.6.2.3.14. Study 14

Data point:	CA 5.2.3/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute inhalation toxicity study four-hour exposure (nose only) in the rat
Report No	710/16
Document No	Not reported
Guidelines followed in study	OECD 403 (1981)
Deviations from current test guideline (OECD 403, 2009)	MMAD slightly exceeded the recommended MMAD. Limit test performed on 5 animals per sex. The test substance batch is not reported. Body weight measured at Day 0 and once a week thereafter.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Acceptability/Reliability	**Conclusion GRG:** Study report not available. Monograph (2000): The study is considered acceptable. Category 4a **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written below was prepared by the AGG. The study is considered acceptable but with restrictions (reliable with restrictions) due to MMAD being outside the range specified in the OECD guidance.

Short description of study design and observations:	One single group of ten Sprague-Dawley strain rats (five males and five females) was exposed for 4 hours to an atmosphere containing 5.35 mg/L of the test material (glyphosate technical, purity: 95 %), using a nose-only exposure system. The generated dust had a mass median aerodynamic diameter (MMAD) of 4.4 µm with a GSD of 0.47 µm. At the start of the study the males weighed 216-232g and the females 199-213g. All animals were observed for clinical signs at hourly intervals during the exposure, immediately on removal from the restraining tubes at the end of the exposure, one hour after termination of the exposure and subsequently once daily for 14 days. All rats were weighed before dosing and on days 7 and 14 post exposure. At sacrifice, all animals were subjected to a macroscopic *post mortem* examination.
Short description of results:	No mortality occurred during the study. During exposure, wet fur was common and there were incidents of decreased respiratory rate. On removal from the chamber, hunched posture and piloerection were additionally noted. Three animals showed ptosis and one animal had brown staining of the fur on the head. One hour after completion of exposure, wet fur was no longer evident. On day one following exposure there were still two rats exhibiting hunched posture and/or piloerection. There were no further abnormalities observed for the rest of the study. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no

	pathological findings. The acute inhalation LC_{50} was calculated to be >5.35 mg/L.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered supplementary since the MMAD is outside the range recommended in the OECD guidance. Under the conditions of the study the LC_{50} > 5.35 mg/L air in male and female rats.

B.6.2.3.15. Study 15

Data point:	CA 5.2.3/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate ([redacted]): Acute inhalation toxicity in rats
Report No	[redacted]94-403/R
Document No	Not reported
Guidelines followed in study	OECD 403 (1981)
Deviations from current test guideline (OECD 403, 2009)	MMAD not measured. Not tested up to recommended limit concentration (2.876 mg/L). Limit test performed on 5 animals per sex instead of 3. Body weight measured at Day 0 and once a week afterwards. Mode of exposure not stated.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP is claimed but no check from the competent authority was provided in the study report.
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered unacceptable as it is not clear the study was performed according to GLP, the limit concentration is too low and the MMAD is not measured and the exposure mode is not described.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in rats when exposed for a single 4-hour period at nominal concentrations of 0, 1.138 and 2.876 mg/L and measured concentrations of 0, 0.653 and 1.538 mg/L, respectively.

No mortality occurred during the study. No clinical signs were observed. There was no effect on body weight gain. The gross necropsy and histopathological evaluation conducted at termination of the study demonstrated no pathological and histopathological findings.

The acute inhalation LC_{50} was calculated to be >2.876 mg/L air (nominal).

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification: Glyphosate

Description:	White crystalline powder
Lot/Batch #:	36300892
Purity:	97.2%
Stability of test compound:	Not reported

2. **Vehicle and/ or positive control:** Water/ None

3. **Test animals:**

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	No data
Sex:	Males and females
Weight at dosing:	Males: 190 – 200 g; Females: 150 – 160 g
Acclimation period:	One week
Diet/Food:	Standard Altromin rodent chow (except during the exposure period)
Water:	Fresh water, *ad libitum* (except during the exposure and the 4-hours post-exposure period)
Housing:	Groups of five animals in steel-Macrolon III cages on daily changed woodchips
Environmental conditions:	Temperature: 20 ± 2 °C
	Humidity: 45 – 70%
	Air changes: 10/ hour
	12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

Test atmosphere generation:

The various aquaeous concentrations of glyphosate were maintained in homogenous state with a magnetic stirrer and released to a TUR-USI 50 therapeutic ultrasound generator at a rate providing complete vaporization.

Exposure chamber conditions:

The amounts of aerosol were measured at hourly intervals. The aerosol was introduced into the respiratory zone of the animals via the glass cone of the inhalation chamber. At hourly intervals 5 minute breaks were inserted meanwhile rats were observed for clinical signs and gravimetric measurements of the product were performed.

Particle size distribution:

No particle size distribution measurement was performed.

Animal assignment and treatment:

Three groups of five rats per sex received the test material at a dose level (nominal) of 0, 1138 and 2876 mg/m^3 via inhalation for 4 hours. The measured concentrations were 0, 653 and 1538 mg/m^3. During exposure symptoms could be observed only on a head region. Clinical observations were made immediately after dosing, at one and four hours postdose and once daily for 14 days. Individual body weights were recorded before treatment and subsequently at weekly intervals. At termination (day 14), all surviving rats were sacrified and necropsied. Gross changes in the thoracic and abdominal organs as well as organ weights were recorded for brain, heart, thymus, stomach, spleen, lung, liver, kidneys, adrenal glands and testes and epididymis. Parallel with necropsies of rats organ fragments from lung, trachea, liver and kidney were sampled from all 20 rats of the

control and high dose groups for histology. The histological changes were classified as slight, moderate and severe lesions. Significance of lesions attributable to product was controlled with the Fisher's exact test.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No clinical signs were observed neither during the exposure nor after the exposure up to the end of the observation period.

C. BODY WEIGHT

No statistically significant differences were noted in body weights. All animals gained the expected body weight.

D. ORGAN WEIGHT

The absolute and relative organ weights were not affected significantly by the test substance.

E. NECROPSY AND HISTOPATHOLOGY

No pathological findings were noted at necropsy.

No statistically significant histopathological lesions were observed. Males of the low dose group revealed a higher weight of the right testes.

III. CONCLUSIONS

Exposure to a mean concentration up to a nominal concentration of 2.876 mg/L did not result in any mortality. Thus, the acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 2.876 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline. No information on the mode of exposure are reported. The tested concentrations are below the recommended limit concentration and no MMAD is provided. Based on these deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is > 2.876 mg/L air.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered unacceptable as it is not clear the study was performed according to GLP, the exposure mode is not described, the limit concentration is too low and the MMAD is not measured. Therefore, no conclusion can be drawn.

B.6.2.3.16. Study 16

Data point:	CA 5.2.3/016
Report author	[redacted]
Report year	1994
Report title	Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat
Report No	545/39

Document No	Not reported
Guidelines followed in study	OECD 403 (1981), Method B2
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex. Body weight measured at Day 0 and once a week afterwards. These deviations did not affect the outcome of the study.
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

The test substance, glyphosate Premix containing 62.2% as glyphosate isopropylamine salt (46.1 % as glyphosate), was evaluated for its acute inhalation toxicity potential, as a 70 % formulation with distilled water, by exposing a single group of ten Sprague-Dawley strain rats (five males and five females) to an aerosol atmosphere of 4.24 mg/L for four hours using a nose only exposure system. No mortality occurred during the study. Common abnormalities noted on the day of exposure were wet fur, hunched posture and piloerection. No similar abnormalities were observed at later time points until the end of the observation period. No mortality was observed. There was no effect on body weight gain. Six animals showed a dark area or multiple dark foci on the lungs at necropsy but no other abnormalities were detected.

The acute inhalation LC50 was determined to be > 4.24 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate Premix
Description:	Pale yellow liquid
Lot/Batch #:	290-JaK-146-4
Purity:	62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate
Stability of test compound:	Stable at room temperature

2. **Vehicle and/ or positive control:** Vehicle: distilled water (the test material was a 70 % formulation in distilled water)
3. **Test animals:**

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	No data (youg adult)
Sex:	Males and females
Weight at dosing:	Male: 200 – 213g; Female: 190 – 203g
Acclimation period:	At least 5 days
Diet/Food:	Rat and Mouse Expanded Diet No. 1 (Special Diets Services Limited, Witham, Essex, UK), *ad libitum* (except during exposure)
Water:	drinking water, *ad libitum* (except during exposure)
Housing:	In groups of 5 animals per cage in solid floor, polypropylene cages, with sawdust bedding

Environmental conditions:	Temperature:	19 – 23 °C
	Humidity:	58 – 64%
	Air changes:	15/hour
	12 hours light/dark cycle	

B. STUDY DESIGN AND METHODS

In life dates: 1994-03-29 to 1994-05-10

Test atmosphere generation:

In order to facilitate aerosolization the test material was diluted with distilled water (7 parts test material:3 parts distilled water). The test atmosphere was generated using a glass concentric jet nebuliser (Radleys, Saffron Walden, Essex).

Exposure chamber conditions:

The cylindrical exposure chamber had a volume of approximately 30 L. The concentration within the exposure chamber was controlled by adjusting the rate of the infusion pump and the air flow rate through the chamber. The extract from the exposure chamber passed through a scrubber trap and was connected with a high efficiency filter to a metered exhaust system. Prior to the start of the study test material atmospheres were generated within the exposure chamber. Chamber concentrations were measured at regular intervals during the exposure periods. A glass fibre filter was placed in a filter holder and temporarily sealed in a port in the exposure chamber in the animals breathing zone. Chamber air was drawn through the filter at a measured rate using a vacuum pump for a suitable time period.
Each filter was weighed before sampling then dried and weighed again in order to calculate by difference the weight of collected material. The difference in the two weights divided by the volume of atmosphere sampled was representative of the chamber concentration. Based on the results of the preliminary work these figures were adjusted for moisture content (29.5 %).

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated three times during the exposure period using a Cascade Impactor. The results were as follows:

Table 6.2.3.16-22: Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat [redacted] 1994): Details of test atmosphere

Mean achieved actual concentration	MMAD	GSD	Respirable amount with particle size ≤1 µm
(mg/L air)	(µm)		(%)
4.24 ± 0.49	1.1	0.57	45.3

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 1.1 µm. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 0.57. Every effort was made to vary the combination of airflow settings with test material input rate to achieve maximum concentrations and optimum particle size distribution.

Animal assignment and treatment:

One group of 5 male and 5 female rats was exposed to an atmosphere containing 4.24 mg/L of the test material, using a nose only exposure system, for a period of 4 h. All animals were observed for clinical signs at hourly intervals throughout the exposure period, on removal from the restraining tubes at the end of the exposure, one day after termination of the exposure and thereafter once daily for 14 days. Individual body weights were

recorded on the day of exposure, days 7 and 14. All animals were subjected to full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity. The lungs of animals showing abnormalities at necropsy were retained and preserved in 10 % buffered formalin.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

During exposure wet fur was observed as a common alteration in any animal after exposure. On removal from the chamber hunched posture and pilo-erection were additionally noted in all animals. Wet fur was no longer evident one hour after completion of exposure whereas hunched posture and piloerection was still observed one hour after chamber removal. On day one following exposure and for the rest of the study no abnormalities were detected.

The clinical signs are summarized in table below.

Table 6.2.3.16-23: Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat [redacted] 1994): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Hours During exposure			On removal from chamber (4 hours)	On-hour Post-exposure	Post-exposure Day				
			1	2	3			1	2	3	4	5-14
Wet fur	Male	5	3	4	5	5	0	0	0	0	0	0
	Female	5	3	5	5	5	0	0	0	0	0	0
Hunched posture	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0
Piloerection	Male	5	0	0	0	5	5	0	0	0	0	0
	Female	5	0	0	0	5	5	0	0	0	0	0

C. BODY WEIGHT

No adverse effects on body weight gain were noted.

D. NECROPSY

Six animals showed a dark area or multiple dark foci on the lungs at necropsy but no other abnormalities were detected. The necropsy findings are summarized in table below.

Table 6.2.3.16-24: Glyphosate technical: Glyphosate premix: Acute inhalation toxicity study four-hour exposure (nose only) in the rat ([redacted] 1994): Necropsy findings

Macroscopic observations	Sex	No. of animals exposed	No. of animals with present finding
Lungs: dark area	Male	5	1
	Female	5	1

Lungs: multiple dark foci	Male	5	0
	Female	5	4

III. CONCLUSIONS

Nose-only exposure to a mean concentration of 4.24 mg/L with a MMAD of 1.1 µm did not result in any mortality. Thus, the acute inhalation LC_{50} of the test substance glyphosate Premix containing 62.2 % as glyphosate isopropylamine salt (46.1 % as glyphosate) after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 4.24 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >4.24 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and the LC_{50} > 4.24 mg/L air in male and female rats. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.3.17. Study 17

Data point:	CA 5.2.3/017
Report author	[redacted]
Report year	1991
Report title	Acute inhalation toxicity study with glyphosate technical in Wistar rats
Report No	[redacted]877.AIN
Document No	Not reported
Guidelines followed in study	OECD 403 (1987)
Deviations from current test guideline (OECD 403, 2009)	MMAD not calculated. Exposure concentration below recommended limit dose (0.64 mg/L (maximum attainable concentration)). Limit test performed on 5 animals per sex instead of 3. Body weight measured at Day 0 and once a week afterwards. Individual data on clinical observations and necropsy are not reported. Instead of nose-only exposure the animals whole body exposure chambers were used which is considered worst-case.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered unacceptable due to the low concentration, the limited data and that the MMAD is not calculated.

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential, by exposing groups of ten Wistar strain rats (five males and five females) to an aerosol atmosphere of 0 and 0.644 mg/L for 4 hours by whole body exposure. The test substance was dissolved in 10 % v/v of diethyleamine in distilled water and atomised to generate an aerosol. All animals were observed for clinical signs during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). All rats were weighed before dosing and on days 7 and 14 post exposure. At sacrifice, all animals were subjected to gross necropsy.
No mortality occurred during the study. During exposure, nasal irritation was observed in many rats. All rats were normal by 24-hours post exposure. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings.

The acute inhalation LC_{50} was calculated to be > 0.644 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glyphosate Technical
Description:	White ordorless crystals
Lot/Batch #:	60 (code: FSG 03090 H/05 March 90)
Purity:	96.8 %
Stability of test compound:	July 1992

2. **Vehicle and/or positive control:** Vehicle: 10% v/v of diethylamine in distilled water

3. **Test animals:**

Species:	Rat
Strain:	Wistar
Source:	[redacted]
Age:	12 weeks
Sex:	Males and females
Weight at dosing:	Males: 140 – 280g; Females: 136 – 240g
Acclimation period:	At least one week
Diet/Food:	Standard "Gold mohur" brand pelleted rat feed (M/s Lipton India Ltd., Bangalore, India), *ad libitum* (except during exposure)
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum* (except during exposure)
Housing:	In groups of 5 animals per cage in standard polypropylene rat cages (size: L 430 x W 270 x H 150 mm) during preexposure. Individual polypropylene cages (size: L 290 x W 220 x H 140 mm) with stainless steel top grill and steam sterilized clean paddy husk bedding during post exposure.
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 67 ± 6% Air changes: 10 – 15/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No data

Test atmosphere generation:

The test substance was dissolved in 10 % v/v of diethyleamine in distilled water to receive a test substance concentration of 15 % (w/v). The solution was atomised at 1.2 kg/cm^2 pressure and an injection rate of 0.4 ml/min to generate the aerosol.

Exposure chamber conditions:
A whole-body exposure chambers consisting of rectangular stainless steel/glass chamber pyramidal at the top and the bottom was used. The exposure chamber had a volume of 0.5 m^3. Two atomizers were used per chamber

with an injection rate of 0.4 mL/min. The output of the two atomizers was delivered to one chamber to attain high concentration of test material in the chamber. The chamber air was sampled once per hour at 15 L/min and the concentration in the air was determined by the colorimetric method of Glass (Glass, R.L. 1981).
No further details on exposure chamber conditions are available.

Particle size distribution:
No data on MMAD and GSD is available. Particle size of mist generated by spraying as measured by microscopic sedimentation analyser is shown in the table below.

Table 6.2.3.17-25: Acute inhalation toxicity study in Wistar rats [redacted] 1991): Details of test atmosphere

Atomizer	Concentration of formulation	Median particle size
	(%)	(µm)
1	50	1.42 ± 0.35
	100	1.22 ± 0.18
2	50	0.95 ± 0.08
3	50	1.34 ± 0.21
4	50	1.34 ± 0.30

No further information available (assignment of atomiser to exposure group not provided)

Animal assignment and treatment:
Two groups of 5 male and 5 female rats was exposed for 4 hours to an atmosphere containing 0 and 0.644 mg/L of the test material (maximum attainable concentration), using a whole body exposure system. All animals were observed for clinical signs during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). All rats were weighed before dosing and on days 7 and 14 post exposure. At sacrifice, all animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

Nasal irritation was observed in few rats of the control group and most rats of the test group during exposure. All rats were normal by 24-hours post exposure.

C. BODY WEIGHT

No adverse effects on body weight gain were noted except for two control rats which had lost weight on day 14.

D. NECROPSY

No gross necropsy observations were noted in any of the animals.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 0.644 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not performed in accordance with the current OECD 403 guideline (2009). The tested concentration is far below the recommended limit concentration and no MMAD is provided. Based on these deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is > 0.644 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
The study is considered unacceptable due to the low concentration, the limited data and that the MMAD is not calculated. Therefore, no conclusion could be drawn. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.3.18. Study 18

Data point:	CA 5.2.3/018
Report author	[redacted]
Report year	1989
Report title	Glyphosate technical: Acute Inhalation Toxicity Study In Rats (Limit Test)
Report No	5993
Document No	Not reported
Guidelines followed in study	OECD 403 (1981), EPA Guidelines, subdivision F, 81-3
Deviations from current test guideline (OECD 403, 2009)	No MMAD calculated (mean measured particle size: 22.5 µm). Limit test performed on 5 animals per sex. Analytical purity of test material not provided
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered unacceptable due to the particle size and the missing information on the MMAD

The test substance, glyphosate technical, was evaluated for its acute inhalation toxicity potential in rats when exposed by snout-only exposure for a single continuous 4-hour period to an actual concentration of 4.98 ± 0.12 mg/L with a mean measured particle size of 22.5 µm.
No mortality occurred during the study. No clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings.

The acute inhalation LC_{50} was calculated to be >4.98 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glyphosate Technical
Description: Fine white powder
Lot/Batch #: 206-JAK-25-1

Purity:	No data (certificate of analysis not available) (Purity: 98.6 % according to CA 5.2.1/025)
Stability of test compound:	No data

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	No data
Sex:	Males and females
Weight at dosing:	118 – 147 g
Acclimation period:	No data
Diet/Food:	Rat and Mouse (Modified) No. 1 Diet SQC Expanded (Special Diets Services Limited, Stepfield, Witham, Essex), *ad libitum* (except during exposure)
Water:	tap water, *ad libitum* (except during exposure)
Housing:	In groups of 1-2 animals per cage in suspended stainless steel mesh cages on absorbent paper.
Environmental conditions:	Temperature: 20 – 25 °C Humidity: 50-66% Air changes: no data 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1989-08-10 to 1989-08-25

Test atmosphere generation:
The test atmosphere was generated using an Aerostyle dust generator.

Exposure chamber conditions:
The cylindrical exposure chamber had a volume of approximately 41.5 L. The exposure system was truly dynamic, incorporating a single pass of the freshly generated material. The particles dispersed throughout the chamber and exited through the base to a filtered vacuum line. Chamber air flow rates were monitored continuously and the values recorded at 30 min intervals. Air flow to the chamber was maintained at 30 L/min and the extract flow at 32 L/min. Chamber concentrations were measured at regular intervals during the exposure periods. The gravimetric method used employed pressed glass fibre filters (Whatman GF/B) placed in a filter holder. The conical input side of the holder was positioned and temporarily sealed in a port in the exposure chamber at the animals' breathing zone. Chamber air was drawn through the filter at a measured rate of 1.0 L/min using a vacuum pump. The air flow during each sample was controlled by a critical orifice and timed for a suitable period.

Each filter was weighed before and after sampling in order to calculate by difference the weight of collected material. The chamber concentration was estimated by further calculation using the sample air volume.

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated twice during the exposure period using a Marple (Model 296) Cascade Impactor (Anderson Samplers Inc., Atlanta, Georgia, USA). The results were as follows:

Table 6.2.3.18-26: Glyphosate Technical: Acute Inhalation Toxicity Study In Rats (Limit Test) [redacted] 1989): Details of test atmosphere

Actual concentration (gravimetric method)	Mass mean diameter	GSD	Respirable amount particle size ≤ 3.5 µm
(mg/L air)	(µm)		(%)
4.98 ± 0.12	22.5	4.6	10

GSD = geometric standard deviation

The generated dust had a mass mean diameter of 22.5 µm. The Geometric Standard Deviation (GSD) of the mass mean diameter was calculated as 4.6. The percentage of particles with a size < 3.5 µm was 10 % by weight.

Animal assignment and treatment:

One group of 5 male and 5 female rats each was exposed to an atmosphere containing 4.98 mg/L of the test material, via the inhalation route, by snout-only exposure for a single continuous 4 h period. All rats were observed for clinical signs at frequent intervals throughout the exposure period, for the first 1-2 h post dosing and thereafter at least once daily during the subsequent 14 day observation period. All rats were weighed immediately before dosing and on Days 2, 3, 4, 7, 10 and 14 post exposure. Each rat was examined externally prior to opening the abdominal and thoracic cavities. Any gross lesions observed were recorded in descriptive terms, including location(s), size (mm), colour and number. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity. All organs were examined in situ. The lungs of each animal were removed and weighed to allow calculation of lung:body weight ratio.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

All animals were slightly subdued after dosing but showed normal behaviour on Day 1 and throughout the remainder of the observation period.

C. BODY WEIGHT

No adverse effects on body weight gain were noted.

D. NECROPSY

There were no findings attributable to treatment with glyphosate technical noted during macropathological examination of the animals.
The lung to body weight ratios for all animals were considered to be within normal limits.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 4.98 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline. No MMAD was calculated, the mean measured particle size was 22.5 µm. Further, no analytical purity of the test material is provided. Based on these deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is >4.98 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered unacceptable since the MMAD is not reported and the GSD is outside the range specified by the OECD guidance. Therefore, the study is considered to be unacceptable. Under the study conditions the LC_{50} > 4.98 mg/L air in male and female rats.

B.6.2.3.19. Study 19

Data point:	CA 5.2.3/019
Report author	[redacted]
Report year	1989
Report title	4-hour acute inhalation toxicity study with glyphosate technical in rats
Report No	238105
Document No	PRO426
Guidelines followed in study	OECD 403 (1981)
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex. MMAD and GSD not specifically reported
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Groups of five Albino Wistar rats per sex were exposed to nebulised test substance (glyphosate salt (batch: 21/39), IPA with a purity of 62 % in water equivalent to 46% (w/w) of N-phosphonomethylglycine acid) for 4 hours via a nose-only exposure system. Animals were exposed to doses of 4.1, 4.42 and 6.49 mg/L air. Clinical signs and mortality were noted one per hour during and once or twice daily respectively following each exposure over a 15-day or 16-day observation period. Body weights were recorded prior to exposure and weekly thereafter. All animals were necropsied.
Short description of results:	A specific MMAD is not reported, but it is stated that the MMAD is 3µm or less. No mortality occurred during the study. During exposure, nose bleeding and ruffled fur were observed. No clinical signs were observed from the day following exposure up to the end of the observation period. No treatment-related effects on body weights were observed and no gross macroscopic changes were found at terminal necropsy The acute inhalation LC_{50} was calculated to be >6.49 mg/L.
Reasons why the study report is not available for submission	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance" (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL

Assessment and conclusion by RMS:
Contrary to the previous conclusion (RAR, 2015) the study is considered acceptable but with restrictions (reliable with restrictions). The MMAD and GSD are not specifically reported although the MMAD is stated to be 3µm or less. Under the study conditions the LC_{50} > 6.49 mg/L air in male and female rats.

B.6.2.3.20. Study 20

Data point:	CA 5.2.3/020
Report author	[redacted]
Report year	1988
Report title	Acute inhalation study of MON-8750 technical

Report No	[redacted] 87-228
Document No	Not reported
Guidelines followed in study	Not reported
Deviations from current test guideline (OECD 403, 2009)	Limit test performed on 5 animals per sex. MMAD is slightly outside the range recommended in the OECD guidance. The limit concentration is too low and instead of nose-only exposure the animals were placed in whole body exposure chambers for the experiment, which is considered worst-case. No certificate of analysis was included in the study report.
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) due to the low limit concentration and MMAD outside the recommended range

The test substance, glyphosate technical (MON 8750 Technical), was evaluated for its acute inhalation toxicity potential, by exposing a single group of ten Sprague-Dawley strain rats (five males and five females) to an aerosol atmosphere of 1.9 mg/L for four hours by whole body exposure. Exposure was followed by a 14-day observation period and subsequent necropsy. No deaths occurred as a result of the exposure, which was performed at the highest attainable concentration. due to the placement of the animals within the chamber, observations during exposure were difficult to make; however, of the animals visible, only hypoactivity was noted. Animals appeared normal immediately after exposure. The only notable observation during the post-exposure period was red/brown perinasal encrustation noted on post-exposure Days 1 and 2. Mean body weight of the animals increased throughout the study period. There were no gross necropsy observations noted at the terminal sacrifice.

The acute inhalation LC_{50} was determined to be > 1.9 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	MON 8750 Technical
Description:	White powder
Lot/Batch #:	XLH-270
Purity:	85.52 %
Stability of test compound:	Not reported

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	Sprague-Dawley
Source:	[redacted]
Age:	9 weeks

Sex:	Males and females
Weight at dosing:	Male: approx. 322g; Female: approx. 214g
Acclimation period:	20 days (arrival: 1987-11-03; first exposure: 1987-11-23)
Diet/Food:	Purina Mills RODENT CHOW No. 5002 (Purina Mills Inc., St. Louis, MO), *ad libitum* (except during exposure)
Water:	Sodium zeolite-conditioned St. Louis public water supply, *ad libitum* (except during exposure)
Housing:	Indivudual suspended stainless steel mesh cages, over paper bedding
Environmental conditions:	Temperature: 68 – 76 °F (approx. 20 - 24 °C)
	Humidity: 35 – 60 %
	Air changes: No data
	12 hours' light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1987-11-23 to 1987-12-7

Test atmosphere generation:

The test atmosphere was generated using a fluidized-bed dust generator.

Exposure chamber conditions:

Animals were exposed in a stainless steel chamber with a pyramidal top and bottom. The concentration within the exposure chamber was controlled by regulating the air flow rate through the dust generator. The chamber air flow, temperature and relative humidity was monitored continuously and recorded approximately every 30 minutes. Chamber concentrations were measured at regular intervals during the exposure periods. Approximately 10 L of test atmosphere was drawn at a known rate a glass fibre filter (25mm Gelman type A/E). Filter was weighed prior to and following the sampling period to determine the net weight of dust collected after drawing a known volume of air from the chamber. The nominal concentration was calculated once for each exposure by determining the total amount of test material delivered to the chamber and dividing this amount by the total air volume passing through the chamber.

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated once during the exposure period using a Andersen Cascade Impactor. The results were as follows:

Table 6.2.3.20-27: Acute inhalation study of MON-8750 Technical [redacted] 1994): Details of test atmosphere

Mean analytical concentration (gravimetric)	MMAD	GSD	Respirable amount particle size ≤10 µm
(mg/L air)	(µm)		(%)
1.9	4.2	1.8	92.5

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

The generated dust had a mass median aerodynamic diameter (MMAD) of 4.2 µm. The Geometric Standard Deviation (GSD) of the MMAD was calculated as 1.8.

Animal assignment and treatment:

One group of 5 male and 5 female rats were exposed to an atmosphere containing 1.9 mg/L of the test material for 4 hours by whole body exposure. All animals were observed for clinical signs at hourly intervals throughout the exposure period, however the placement of cages allowed only limited observation of animals, immediately following exposure and once daily (normal work days only) during 14-day observation period following exposure. The animals were observed twice daily for mortality and moribundity. Individual body weights were recorded on the day of exposure, days 2, 7 and 14. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded.

II. RESULTS

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

During exposure hypoactivity was observed. No clinical signs were detected immediately after exposure. Red/Brown perinasal encrustation was observed in two males on Days 1-2 during the post-exposure period.

For the rest of the study, Days 3-14, all animals appeared normal. No abnormalities were detected in females.

The clinical signs are summarized in table below.

Table 6.2.3.20-28: Acute inhalation study of MON-8750 Technical [redacted] 1994): Clinical observation data

Clinical sign	Sex	No. of animals exposed	Post-exposure Day													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Red/Brown perinasal encrustation	Male	5	2	2	0	0	0	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0

C. BODY WEIGHT

No adverse effects on body weight gain were noted.

D. NECROPSY

No gross necropsy observations were noted in any of the animals.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 1.9 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed in accordance to OECD 403 guideline and GLP. The MMAD and the applied concentration were in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable. Based on the study results, the acute inhalation LC_{50} is >1.9 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered acceptable but with restrictions (reliable with restrictions) since the limit concentration is too low, and the MMAD is outside the range specified in the OECD guidance. Therefore, the study is considered to be acceptable but with restrictions (reliable with restrictions). Under the study conditions the LC_{50} > 1.9 mg/L air in male and female rats.

B.6.2.3.21. Study 21

Data point:	CA 5.2.3/021
Report author	[redacted]
Report year	1987
Report title	Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats
Report No	[redacted] 6582
Document No	[redacted] 86105
Guidelines followed in study	None
Deviations from current test guideline (OECD 403, 2009)	Exposure concentration slightly below recommended limit dose (1.3 mg/L (maximum attainable concentration of the herbicide containing approx. 54 % isopropylamine salt of glyphosate)). Limit test performed on 5 animals per sex. Body weight measured at Day 0, 2, 7 and 14. instead of nose-only exposure the animals were placed in whole body exposure chambers for the experiment, which is considered worst-case. Individual data is not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 2a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the low concentration and the lack of individual animal data.

The test substance, Rodeo® Herbicide,containing 53.8 % Isopropylamine salt of glyphosate was evaluated for its acute inhalation toxicity potential, by exposing a single group of ten Sprague-Dawley strain rats (five males and five females) to an aerosol/vapour atmosphere of 1.3 mg/L (maximum attainable concentration) for four hours by whole body exposure. Exposure was followed by a 14-day observation period and subsequent necropsy. One female rat died. Focal and/or generalized loss of hair developed in some animals during the observation period. There was a decrease in group mean body weight on post-exposure Day 2 relative to pre-exposure mean weight. On Days 7 and 14 group mean body weight was increased. Gross necropsy examination of animals revealed no alterations that were considered related to exposure.

The acute inhalation LC_{50} was calculated to be > 1.3 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification: Rodeo® Herbicide
Description: Amber liquid
Lot/Batch #: LHRO-12010 X

Purity:	Stated composition: 53.8 % Isopropylamine salt of glyphosate, 46.2 % Inert
Stability of test compound:	Expiry date: December 1987

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	Sprague-Dawley (Crl: CD®(SD)BR)
Source:	[redacted]
Age:	8 – 10 weeks
Sex:	Males and females
Weight at dosing:	Male: 271 – 286 g; female: 235 – 242 g
Acclimation period:	At least 10 days
Diet/Food:	Purina Certified Rodent Chow® (5002), *ad libitum* (except during exposure)
Water:	Sodium zeolite-conditioned St. Louis public water supply, *ad libitum* (except during exposure)
Housing:	Individually in suspended stainless steel mesh cages
Environmental conditions:	Temperature: 68 – 76 °F (22 ± 2 °C) Humidity: 35 – 60% Air changes: Not specified 12-hour light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 1986-07-29 to 1986-08-12

Test atmosphere generation:

The test atmosphere was generated by using a capillary restrictor from a tank to a Laskin-type nebuliser located in the top turret of the exposure chamber.

Exposure chamber conditions:

The exposure chamber was a 300 L New Yorker University-style stainless steel chamber, with a pyramidal top and bottom and a glass window in the door.

The concentration of test material in the chamber was controlled by regulating the pressure in the tank headspace and consequently, the flowrate of the test material into the nebuliser. Chamber airflow, temperature, and relative humidity were monitored continuously and recorded every 30 minutes. Airflow through the chamber ranged from 58.3 to 69.2 L/min during the exposure. The mean chamber temperature was 24 °C and the mean relative humidity was 66 %. Oxygen level was determined once during the exposure using a Draeger tube and was determined to be 19 %.

Test material concentrations in the inhalation chamber were routinely obtained four different times during the exposure at approximate one-hour intervals. The analytical concentration of test material was determined by liquid chromatography. The mean analytical concentration for the exposure was 1.3 mg/L in air that is considered to be the maximum attainable concentration. The nominal concentration of 47 mg/L was calculated by determining the amount of test material delivered to the chamber and dividing this amount by the air volume passing through the chamber. The high nominal/analytical ratio appeared to be due to loss from the exposure atmosphere of the test material aerosol because of impaction on the walls of the chamber, cages or animals.

Particle size distribution:

The particle size distribution of the dispersed material inside the exposure chamber was estimated once during the exposure period using an Andersen Cascade Impactor. The results were as follows:

Table 6.2.3.21-29: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted], 1987): Details of test atmosphere

Mean analytical concentration (gravimetric)	MMAD	GSD	Respirable amount particle size ≤ 10 µm
(mg/L air)	(µm)	(µm)	(%)
1.3	3.1	1.9	97.0

MMAD = mean mass median aerodynamic diameter
GSD = geometric standard deviation

Animal assignment and treatment:

One group of 5 male and 5 female rats were exposed to an aerosol/vapour atmosphere containing 1.3 mg/L of the test material for 4 hours by whole body exposure. All animals were observed during the exposure but placement of the cages allowed only limited observation. Animals were thoroughly examined following exposure and gross signs of toxicity were recorded. During the 14-day observation period the animals were scheduled for examination twice daily for mortality and once daily for gross signs of toxicity. Body weights were obtained prior to exposure (Day 0) and on post-exposure Days 2, 7 and 14. A standard macroscopic examination of the external appearance and of the tissues of the thoracic, abdominal, and cranial cavities was conducted on animals found dead and those sacrificed at the end of the study.

II. RESULTS

A. MORTALITY

Exposure to 1.3 mg/L resulted in mortality of one female on post-exposure Day 3. No further unscheduled death was observed until the end of the observation period.

B. CLINICAL OBSERVATIONS

Observations recorded following the exposure are presented in the table below.

Table 6.2.3.21-30: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted] 1987): Clinical observation data

Exposure Level (mg/L)	Observation	Number Observed		Day observed	
		Male	Female	First	Last
1.3	Labored respiration	0	1	2	2
	Rapid respiration	0	1	2	2
	Yellow/brown nasal discharge	4	5	0	7
	Bloodlike oral discharge	0	1	2	2
	Red ocular discharge	2	1	0	1
	Periocular encrustation	1	1	2	3
	Auricular encrustation	1	0	9	14
	Focal loss of hair	2	2	3	14
	Generalized loss of hair	2	0	4	14
	Red/brown perinasal encrustation	2	1	1	1
	Diarrhea	1	0	2	7
	Emaciated	1	1	2	7

Yellow/brown nasal discharge was observed in most of the exposed animals (8/10) immediately after exposure (Day 0). This sign did not persist - on the day after exposure (Day 1) only 2/10 animals exhibited this observation and on Day 8 this sign was not seen in any animal. Focal and/or generalized loss of hair developed in some animals (5/10) during the 14-day observation period.

C. BODY WEIGHT

On post-exposure Day 2 both males and females revealed decreases in mean body weights, however, mean body weights were increased over initial weights on Days 7 and 14 (see table below).

Table 6.2.3.21-31: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted] 1987): Body weight

Day		**0**	**2**	**7**	**14**	**0**	**2**	**7**	**14**
Sex		**Males**				**Females**			
Exposure level (mg/L)	**Animal No.**	**Body weight [g]**							
1.3	1	286	288	321	362	241	237	240	247
	2	278	276	298	333	235	212	-	-
	3	284	257	264	312	242	234	246	251
	4	282	277	287	320	241	235	255	268
	5	271	270	298	326	236	229	235	245
	Mean	**280**	**274**	**294**	**331**	**239**	**229**	**244**	**253**
	± SD	**± 6**	**± 11**	**± 21**	**± 19**	**± 3**	**± 10**	**± 9**	**± 10**

- = animal dead

D. NECROPSY

Gross necropsy examination revealed alterations in the kidneys, lung, skin, thymus, and uterus (see table below). None of these alterations were considered related to the exposure and are considered findings that are likely due to post mortem changes or are observations frequently seen in animals of this species and strain processed similarly.

Table 6.2.3.21-32: Acute Toxicity of Rodeo® Herbicide Administered by Inhalation to Male and Female Sprague-Dawley Rats [redacted] 1987): Necropsy data

Exposure level [mg/L]	**1.3**	
Sex	**Males**	**Females**
Animals examined	5	4*
Kidney		
- Abnormal color, brown/yellow/tan	1	1
- Focus, brown/yellow/tan	1	0
- Focus, red/purple/black	2	0
Lung		
- Focus	3	3
Skin		
- Alopecia	2	0
Thymus		
- Multiple, purple-red foci	5	3
Uterus		
- Hydrometra	0	1

* No gross necropsy alterations were observed in the dead female.

III. CONCLUSIONS

The acute inhalation LC_{50} of the test substance, Rodeo® Herbicide, containing 53.8 % Isopropylamine salt of glyphosate after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 1.3 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was performed similar to OECD 403 guideline and according to GLP. The maximum attainable concentration of 1.3 mg/L air contained 53.8 % isopropylamine salt of glyphosate. The MMAD and GSD was in the range of the recommended values of the current OECD guideline. Otherwise, only minor deviations to the current valid OECD guideline are present. Therefore the study is acceptable as supportive. Based on the study results, the acute inhalation LC_{50} is >1.3 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
Contrary to the previous evaluation (RAR, 2015) the study is considered acceptable but with restrictions (reliable with restrictions) since the limit concentration is too low and individual animal data was not reported. Therefore, the study is considered to be acceptable but with restrictions (reliable with restrictions). Under the study conditions the LC_{50} > 1.3 mg/L air in male and female rats.

B.6.2.3.22. Study 22

Data point:	CA 5.2.3/022
Report author	[redacted]
Report year	1983
Report title	Report on acute inhalation toxicity in rats (4 hours) of glyphosate (technical)
Report No	Not given
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 403, 2009)	Yes, the following information were not given: Purity and stability of test substance, strain of test animals, acclimation period, vehicle. Age of animals was in the range of 6 - 7 weeks instead of 8 - 12 weeks. Body weights of animals after exposure were not determined. Individual animal data not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered unacceptable since the study is not conducted according to GLP, the limited reporting

The test substance, glyphosate technical (Glycel 4l SL), was evaluated for its acute inhalation toxicity potential, by exposing groups of ten rats (five males and five females) to a nominal aerosol atmosphere of 0, 1.4, 2.3 and 4.5 mg/L for four hours. Nose- and mouth-inhalation route was used. All animals were observed for clinical signs before and at hourly intervals during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). At sacrifice, all animals were subjected to gross necropsy.
No mortality occurred during the study. No toxic symptoms and no remarkable pathological changes were observed in any of the animals exposed to the test material.

The acute inhalation LC_{50} was determined to be > 4.5 mg/L air.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	Glycel 4l SL
Description:	Not specified
Lot/Batch #:	Not specified
Purity:	Not specified
Stability of test compound:	Not specified

2. **Vehicle and/or positive control:** No information provided

3. **Test animals:**

Species:	Rat
Strain:	Not specified
Source:	Breeding colony, not further specified
Age:	6 – 7 weeks
Sex:	Males and females
Weight at dosing:	90 – 100 g
Acclimation period:	Not specified
Diet/Food:	Gold Mohor rat food (Hindustan Lever Limited), *ad libitum* (except during exposure)
Water:	*ad libitum* (except during exposure)
Housing:	In groups of 5 animals per cage in polypropylene cages
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 25 – 75 % Air changes: 12/hour 12 hours light/dark cycle

B. STUDY DESIGN AND METHODS

In life dates: No data

Test atmosphere generation:

The test material was nebulised with compressed air at 10 psi and at the rate of 10 L/min.
No further details on test atmosphere generation are available.

Exposure chamber conditions:

The chamber was exhausted by the use of vacuum pump evaluating 20 L/min. The exhausted air was led passively to the outside of the building and vented. The size of the chamber was 78 L.

The temperature (19 – 25 °C) and humidity (25 – 75 %) inside the exposure chamber was measured continuously and recorded at hourly intervals throughout each exposure period. The oxygen concentration inside the chambers was monitored continuously using oxygen analyser and recorded at hourly intervals. The mean oxygen concentration recorded for all groups was not less than 21 %. The mean diluent air flow rates were similar for all groups.

The absolute exposure chamber atmosphere concentration was measured hourly by withdrawing air from the chamber at the rate of 4.86 L/min on a Whatman 4l filter paper in a filter assembly. The difference in weights of

the paper before and after the exposure gave the weight of the product. The nominal concentration was the weight of test material used divided by the volume of diluent air used. The nominal concentrations were 4.6 mg/L (low dose), 9.1 mg/L (intermediate dose) and 16.7 mg/L (high dose). The overall mean measured concentrations were 1.4 mg/L, 2.3 mg/L and 4.5 mg/L for low, intermediate and high dose, respectively.

No further details on exposure chamber conditions are available.

Particle size distribution:

The particle size of the generated atmosphere of the test material was determined hourly using the method and operations of an Anderson Sampler Mark II. The mass median aerodynamic diameters (MMAD) mean values were 3.8 µm (low dose), 4.0 µm (intermediate dose) and 3.9 µm (high dose).

Animal assignment and treatment:

Four groups of 5 male and female rats were exposed for 4 hours to an atmosphere containing nominal concentrations of 0, 1.4, 2.3 and 4.5 mg/L (maximum attainable concentration) of the test material. Nose- and mouth-inhalation route was used. All animals were observed for clinical signs before, at hourly intervals during the exposure for 4 hours (day 1) and once daily during post exposure (days 2-14). At sacrifice, all animals were subjected to gross necropsy.

II. RESULTS

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
No toxic symptoms were seen in any of the animals.

C. BODY WEIGHT
Body weights were not recorded.

D. NECROPSY
No remarkable pathological changes were seen in any of the animals.

III. CONCLUSIONS

The acute inhalation LC_{50} of glyphosate technical (Glycel 4l SL) after a 4-hour exposure to male and female rats, observed over a period of 14 days was calculated to be greater than 4.5 mg/L.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not performed according to any guideline. No information on GLP status is reported. Due to limited reporting, and minor deviations, the study is considered as supportive. Based on the study results, the acute inhalation LC_{50} is > 4.5 mg/L air after an exposure period of 4 hours.

Assessment and conclusion by RMS:
The study is considered unacceptable since it is not performed according to any guideline or GLP. The reporting is limited, the individual data is not available and the purity of the test substance is not reported. Therefore, no conclusion could be drawn. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4. Skin irritation

B.6.2.4.1. Study 1

Data point:	CA 5.2.4/001
Report author	[redacted]

Report year	2011
Report title	Glyphosate technical - Primary skin irritation study in rabbits
Report No	10/218-006N
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), US EPA OPPTS 870.2500 (1998); EC No 44/2008, B.4 (2008)
Deviations from current test guideline (OECD 404, 2015)	Instead of a semi-occlusive dressing an occlusive dressing was used. Since, an occlusive dressing is considered worst-case and only minimal skin irritation was reported, this deviation is not considered to impact the outcome of the study.
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

In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted glyphosate technical (Batch: 569753 (BX20070911), Purity: 96.3 % w/w). The test substance was applied to an area of the intact shaved flank (2.5 cm × 2.5 cm), under a semi-occlusive dressing. The patch was hold in place with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking. After 4 hours, the dressing was removed, and the skin was flushed with lukewarm tap water to clean the application site. Initially, a single animal was treated. As neither a corrosive effect nor a severe irritant effect was observed after the 1-hour exposure, the test was completed using the two remaining animals.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004 which was based on the Draize scoring system. The scoring of skin reactions was performed 1, 24, 48 and 72-hours after removal of the dressing. 1 and 24-hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study. The individual mean score for the 24, 48 and 72-hour readings were 0.33 for one animal and 0.0 for the remaining animals for erythema and 0.0 for oedema for all animals, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate technical
Description:	Dry white powder
Lot/Batch number:	569753 (BX20070911)
Purity:	96.3 % w/w
Stability of test compound:	Stable under storage conditions (room temperature range < 30 °C), Expiry date: 2011-08-31

2. **Vehicle and/ or positive control:** Not specified
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 12 weeks
Sex:	Male
Weight at dosing:	2995 – 3095 g
Acclimatisation period:	5 days
Diet:	Purina Base – Lap gr. diet (AgribrandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary), *ad libitum*
Water:	Municipal tap water *ad libitum*
Housing:	Individually in metal cages
Environmental conditions:	Temperature: 17 – 20 °C
	Humidity: 30 – 70 %
	Air changes: 15 – 20 / hour
	Photocycle: 12 hours light / dark

4. Test conditions:

Patch site preparation technique:	The back and flanks were clipped (area of approximately 100 cm^2) with an electric clipper approximately 24-hours before treatment.
Patching technique:	Dermal application onto shaved, intact dorsal skin (area 2.5 cm × 2.5 cm). The patch was kept in contact with the skin by a patch with surrounding adhesive hypoallergenic plaster.
Chemical preparation:	Glyphosate Technical was applied undiluted
Chemical application:	0.5 g / animal
Chemical removal:	Skin was flushed with lukewarm tap water

B: STUDY DESIGN AND METHODS:

In-life dates: 2010-11-02 to 2010-11-05

Animal assignment and treatment:

In a primary dermal irritation study, three male, young adult, New Zealand White rabbits were each given a dermal application of 0.5 g of undiluted glyphosate technical (96.3 % w/w glyphosate technical).

Approximately 24-hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area approximately 10 cm × 10 cm. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of glyphosate technical was placed on a surgical gauze pad (approximately 2.5 cm × 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking. The dressing was left in place for 4 hours, after which it was removed, and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible.

As it was suspected that the test item might produce irritancy, a single animal was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals. The animals were checked daily for signs of systemic toxicity and mortality. Body weights were recorded on the day of application and at termination of observations.

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004, which was based on the Draize scoring system (see table below), approximately 1, 24, 48 and 72-hours after the removal of the dressing, gauze patch and test item. The mean score was calculated across three scoring times (24, 48 and 72-hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately.

Skin reaction grading according to Draize criteria used by [redacted] (2011)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation (injuries in depth preventing erythema) reading	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 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animals during the study.

C. BODY WEIGHT

The body weights of the rabbits were considered to be within the normal range of variability.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

The test substance did not elicit any skin reactions at the application site of any animal at any of the observation times (all scores 0). 1 and 24-hours after patch removal, very slight erythema (score 1) was observed in one animal. No signs of irritation were observed in the other treated animals throughout the study.

The individual mean score for the 24, 48 and 72-hour readings were 0.33 for one animal and 0.0 for the remaining animals for erythema and 0.0 for oedema for all animals, respectively. No staining of the treated skin or other alterations or corrosive effects was observed.

As no signs of irritation were observed 72-hours after patch removal, the study was terminated after the 72-hour observation.

Table 6.2.4.1-1 Glyphosate technical - Primary Skin Irritation Study in Rabbits [redacted] 2011): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	00606 (♂)	0	0
	00622 (♂)	1	0
	00620 (♂)	0	0
24-hours	00606 (♂)	0	0
	00622 (♂)	1	0
	00620 (♂)	0	0
48 hours	00606 (♂)	0	0
	00622 (♂)	0	0
	00620 (♂)	0	0
72-hours	00606 (♂)	0	0
	00622 (♂)	0	0
	00620 (♂)	0	0
Individual 24 – 72 h means	**00606 (♂)**	**0.0**	**0.0**
	00622 (♂)	**0.33**	**0.0**
	00620 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of 0.5 g glyphosate technical to intact rabbit skin for 4 hours elicited a very slight erythema (score 1) in one animal after 1 and 24-hours. No dermal observation was recorded in this animal thereafter. No skin reactions at the application site were observed in the other two treated animals at any observation time. The individual mean score for the 24, 48 and 72-hour readings were 0.33 for one animal and 0.0 for the remaining animals for erythema and 0.0 for oedema for all animals, respectively.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable even though an occlusive dressing was used, because this is considered worst-case and only minimal skin irritation was reported. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.11 for erythema and 0.0 for oedema in male rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.2. Study 2

Data point:	CA 5.2.4/002
Report author	[redacted]
Report year	2010
Report title	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Report No	24605
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), EC method B.4. (2004/73/EC), US EPA OPPTS 870.2500 (1998)
Deviations from current test guideline (OECD 404, 2015)	Himalayan rabbits were used instead of New Zealand White rabbits. The number of air changes was not specified. This deviation is not considered to affect the study outcome.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. The study is considered acceptable.

The test substance, glyphosate TC (Batch: 20090506, Purity: 97.3 %), was evaluated for its potential to cause irritant / corrosive effects. In a primary dermal irritation study, three young male Himalayan rabbits were dermally exposed to glyphosate TC. The clipped intact dorsal skin of the trunk was exposed to 0.5 g of the solid test item (area: approximately 6 cm^2), moistened with 0.5 or 1 mL *aqua ad iniectabilia*, for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a non-irritating tape. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

None of three male rabbits exposed for 4 hours to 0.5 g glyphosate TC per animal (semi-occlusive conditions) showed any test item-related changes. The mean for the 24, 48 and 72-hour readings for each animal was 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate TC does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3 %
Water solubility:	Yes
Stability of test compound:	Expiry date: 2011-05

2. **Vehicle and/or positive control:** Purified water for injection

3. **Test animals:**

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 6 – 7 months
Sex:	Male
Weight at dosing:	2.4 – 2.9 kg
Acclimation period:	At least 20 days
Diet/Food:	Commercial diet, ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum*, except during the exposure period
Water:	Tap water, *ad libitum*, except during the exposure period

Housing:	Individually, in cages measuring 380 mm × 425 mm × 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany) before and after the exposure period. During the exposure period the animals were kept singly in restrainers which allowed free movement of the head but prevented a complete body turn.	
Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 – 70 %
	Air changes:	Not reported
	Photocycle:	12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal area of the trunk was closely clipped, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin (6 cm^2). The patch was held in position with non-irritating tape for exposure period duration.
Chemical preparation:	1000 or 2000 mg was mixed with 0.5 or 1 mL *aqua ad iniectabilia* [water for injection] respectively and 750 mg were applied per animal (≈ 0.5 g test item/animal).
Chemical application:	0.5 g glyphosate TC / animal
Chemical removal:	No

B: Study design and methods

In life dates: 2009-10-26 to 2009-11-06

Animal assignment and treatment:

Approximately 24-hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 0.5 g of the test item was applied to the test site (area: approximately 6 cm^2) and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control. Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers. At the end of the exposure time no residual test item had to be removed.

As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours. As neither a corrosive effect nor a severe irritant effect was observed after a 4 hour exposure period, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2010)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATION

The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72-hours after patch removal. None of the three male rabbits showed any significant test item-related lesions at these examination time points.

Table 6.2.4.2-1 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] 2010): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
24-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
48 hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
72-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
Individual 24 – 72 h means	**1 (♂)**	**0.0**	**0.0**
	2 (♂)	**0.0**	**0.0**
	3 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours

was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Glyphosate is not considered a skin irritant.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.4.3. Study 3

Data point:	CA 5.2.4/003
Report author	[redacted]
Report year	2009
Report title	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Report No	24877
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), Commission Directive 2004 B.4 (2004/73/EC), US EPA OPPTS 870.2500 (1998)
Deviations from current test guideline (OECD 404, 2015)	Himalayan rabbits were used instead of New Zealand White rabbits. The number of air changes was not specified. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

In a primary dermal irritation study, three young male adult Himalayan rabbits were dermally exposed to glyphosate TC (Batch: 2009051501, Purity: 96.4 %). The clipped, intact skin was exposed to 0.5 g of the solid test item, moistened with purified water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. The mean for the 24, 48, and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate TC does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification: Glyphosate TC
Description: White solid powder
Lot/Batch #: 2009051501
Purity: 96.4 %

Stability of test compound:	At room temperature in the dark stable Expiry date: 2011-05-15
2. Vehicle and/or positive control:	Purified water for injection
3. Test animals:	
Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 7.5 – 8.5 months
Sex:	Male
Weight at dosing:	2.6 – 3.2 kg
Acclimation period:	At least 20 days
Diet/Food:	Commercial diet, ssniff K-H V2333 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individual housing in cages (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany) before and after the exposure period. During the exposure period the animals were kept singly in restrainers which allowed free movement of the head but prevented a complete body turn.
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 30 – 70 % Air changes: Not specified Photoperiod: 12 hours light / dark cycle
4. Test conditions:	
Patch site preparation technique:	Closely clipping the dorsal area of the trunk, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin. The patch was held with non-irritating tape for exposure period duration.
Chemical preparation:	1000 or 2000 mg was mixed with 0.5 or 1 mL *aqua ad iniectabilia* [water for injection] respectively and 750 mg were applied per animal (≈ 0.5 g test material/animal).
Chemical application:	0.5 g glyphosate TC / animal
Chemical removal:	No

B: Study design and methods

In life dates: 2009-10-15 to 2009-10-23

Animal assignment and treatment:

The test was conducted using three young male adult Himalayan rabbits. The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance was moistened with purified water and applied to the intact skin of the rabbits on an approx. 6 cm^2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed. No residual test item had to be removed.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the scoring system of the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2009)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	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 (raised approx. 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hours readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Table 6.2.4.3-1 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] 2009): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
24-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
48 hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
72-hours	1 (♂)	0	0

	2 (♂)	0	0
	3 (♂)	0	0
Individual 24 – 72 h means	**1 (♂)**	**0.0**	**0.0**
	2 (♂)	**0.0**	**0.0**
	3 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Glyphosate is not considered a skin irritant.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.4.4. Study 4

Data point:	CA 5.2.4/004
Report author	[redacted]
Report year	2009
Report title	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits
Report No	23913
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), EC method B.4. (2004/73/EC), US EPA OPPTS 870.2500 (1998)
Deviations from current test guideline (OECD 404, 2015)	Himalayan rabbits were used instead of albino New Zealand rabbits. The number of air changes was not specified. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

The test substance, glyphosate TC (Batch: 20080801, Purity: 98.8 %), was evaluated for its potential to cause irritant / corrosive effects. In a primary dermal irritation study, three young male Himalayan rabbits were dermally exposed to glyphosate TC. The clipped intact dorsal skin of the trunk was exposed to 0.5 g of the solid test material (area: approximately 6 cm^2), moistened with 0.5 or 1 mL *aqua ad iniectabilia*, for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a non-irritating tape. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

None of three male rabbits exposed for 4 hours to 0.5 g glyphosate TC per animal (semi-occlusive conditions) showed any test item-related changes. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate TC does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8 %
Water solubility:	Yes
Stability of test compound:	Expiry date: 2010-08-01

2. Vehicle and/or positive control: Purified water for injection

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 4 – 5 months
Sex:	Male
Weight at dosing:	3.8 – 4.4 kg
Acclimation period:	At least 20 days
Diet/Food:	Commercial diet, ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum* except during the exposure period
Water:	Tap water, *ad libitum* except during the exposure period
Housing:	Individually in cages measuring 380 mm × 425 mm × 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany) before and after the exposure period. During the exposure period the animals were kept singly in restrainers which allowed free movement of the head but prevented a complete body turn.
Environmental conditions:	Temperature: 20 ± 3 °C
	Humidity: 30 – 70 %
	Air changes: Not specified
	Photoperiod: 12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal area of the trunk was closely clipped, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin. The patch was held in position with non-irritating tape for exposure period duration.
Chemical preparation:	1000 or 2000 mg was mixed with 0.5 or 1 mL *aqua ad iniectabilia* [water for injection] respectively, and 750 mg were applied per animal (≈ 0.5 g test item / animal).

Chemical application: 0.5 g glyphosate TC / patch and animal

Chemical removal: No

B: Study design and methods

In life dates: 2009-02-04 to 2009-02-13

Animal assignment and treatment:

Approximately 24-hours before the test, the fur was removed by closely clipping the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. Only animals with healthy intact skin were used.

A dose of 0.5 g of the test item was applied to the test site (area: approximately 6 cm^2) and then covered with a gauze patch. The patch was held in contact with the skin with non-irritating tape for the duration of the exposure period. The surrounding untreated skin served as a control. Exposure time was 4 hours. During the exposure the animals were kept in comfortable restrainers. At the end of the exposure time no residual test item had to be removed.

As it was expected that the test item would not produce any severe irritancy or corrosion, the test was started using at first only one animal, receiving a single patch for an exposure period of 4 hours. As neither a corrosive effect nor a severe irritant effect was observed after a four-hour exposure period, the test was completed using two additional animals, each with one patch only, for an exposure period of 4 hours.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2009)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATION

The reactions of the intact skin were evaluated at 60 minutes and then at 24, 48 and 72-hours after patch removal. None of the three male rabbits showed any significant test item-related lesions at these examination time points.

Table 6.2.4.2-1 Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] 2009): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
24-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
48 hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
72-hours	1 (♂)	0	0
	2 (♂)	0	0
	3 (♂)	0	0
Individual 24 – 72 h means	**1 (♂)**	**0.0**	**0.0**
	2 (♂)	**0.0**	**0.0**
	3 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Glyphosate is not considered a skin irritant.
The conclusion is the same as from the previous evaluation (RAR, 2015).

B.6.2.4.5. Study 5

Data point:	CA 5.2.4/005
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Acute Dermal Irritation Study in Rabbits
Report No	12173-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.2500
Deviations from current test	Step-wise approach by initial testing in one animal was not performed and

guideline (OECD 404, 2015)	three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Water solubility was not documented. Humidity was in the range of 43 – 92 % instead of 30 – 70 %. The stability of the test chemical is not reported. These deviations did not compromise the negative study outcome.
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

The test substance, glyphosate tech grade mixed 5-batch (Batch: 080704-1 thru 5, Purity: 96.4 %), was evaluated for its potential to cause irritant / corrosive effects. Therefore, a primary dermal irritation study was conducted on three New Zealand White rabbits (one male, two females).

The clipped intact dorsal skin of the trunk was exposed to 0.5 g of the solid test item (2.5 × 2.5 cm), moistened with 0.2 mL deionised water, for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a strip of non-irritating adhesive tape. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

None of three rabbits exposed for 4 hours to 0.5 g glyphosate tech grade mixed 5-batch (semi-occlusive conditions) showed any test item-related changes. The mean individual score for the 24, 48 and 72-hour readings was 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate tech grade mixed 5-batch does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Tech Grade; Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.40 % (per certificate of analysis dated 2008-10-17)
Stability of test compound:	A second certificate of analysis dated 2009-01-08 with a purity value of 96.71 % demonstrated that the test substance was stable for the duration of the test.

2. **Vehicle and/or positive control:** Deionised water

3. **Test animals:**

Species:	Albino rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approx. 3 months
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Male: 2.000 kg; Females: 2.600 kg
Acclimation period:	5 days

Diet/Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily	
Water:	Tap water, *ad libitum*	
Housing:	Individual housing in suspended, wire bottom, stainless steel cages	
Environmental conditions:	Temperature:	17 – 22 °C
	Humidity:	43 – 92 %
	Air changes:	10 – 12 / hour
	Photoperiod:	12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal area of the trunk was clipped, 24-hours before application to expose at least 8 × 8 cm.
Patching technique:	Dermal application onto shaved, intact dorsal skin (2.5 × 2.5 cm) and the patch was held in position with a strip of non-irritating adhesive tape for exposure period duration (semi-occlusive dressing).
Chemical preparation:	0.5 g glyphosate was moistened with 0.2 mL of deionised water
Chemical application:	0.5 g / animal
Chemical removal:	Skin was washed with room temperature tap water

B: Study design and methods

In life dates: 2008-11-11 to 2008-11-14

Animal assignment and treatment:

Each animal was prepared on the day prior to treatment by clipping the dorsal area of the trunk free of hair to expose an area at least 8 × 8 cm. Only those animals with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site while the contralateral intact site served as a control site.

On Day 0, 0.5 g of test substance moistened with 0.2 mL of deionised water was applied to each test site and covered with a 4 ply, 2.5 × 2.5 cm surgical gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each animal was loosely wrapped with a semi-permeable dressing (orthopaedic stockinette) which was secured on both edges with strips of tape to retard evaporation of volatile substances and to prevent possible ingestion of the test substance. After 4 hours, the patches and wrappings were removed. The test sites were gently washed with room temperature tap water and a clean cloth to remove as much residual test substance as possible.

Skin reactions were assessed approximately 1, 24, 48 and 72-hours after removal of the patch according to the Draize scheme (see table below).

Skin reaction grading according to Draize criteria used by [redacted] (2009)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef 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 (raised approximately 1 mm)	3

Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No data were reported.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No data on necropsy were reported.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.3-1 Glyphosate – Acute Dermal Irritation Study in Rabbits [redacted] 2009): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
24-hours	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
48 hours	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
72-hours	3228 (♂)	0	0
	3227 (♀)	0	0
	3229 (♀)	0	0
Individual 24 – 72 h means	**3228 (♂)**	**0.0**	**0.0**
	3227 (♀)	**0.0**	**0.0**
	3229 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, no report of water solubility and humidity in the range of 43 – 92 % instead of 30 – 70 % are noted. Nevertheless, these deviations did not compromise the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.6. Study 6

Data point:	CA 5.2.4/006
Report author	[redacted]
Report year	2008
Report title	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical
Report No	[redacted] 3996.311.476.07
Document No	Not reported
Guidelines followed in study	OECD 404 (2002)
Deviations from current test guideline (OECD 404, 2015)	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

The acute dermal irritation/corrosion potential of glyphosate technical (Batch: 20070606, Purity: 98.05 %) was evaluated in three New Zealand White rabbits (females). The test was initially conducted using one single rabbit. Because no dermal reaction was observed in the initial test, two additional animals were tested to confirm the response. A moistened gauze patch containing 0.5 g of the undiluted test item was applied to the clipped back (approx. 6 cm^2) of each animal. The patch was held in contact with the skin by an adhesive and a non-irritating tape. After the 4-hour semi-occlusive exposure period, the patches were removed, any residual test item was washed using physiological saline solution, and the animals were examined at approximately 1, 24, 48 and 72-hours to verify the erythema, eschars, and oedema formation, and for behavioural and clinical alterations. Adjacent untreated shaved areas of the skin were used as the negative control.

The test item applied on the skin of the rabbits did not cause any dermal irritation. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. No treatment-related behavioural or clinical alterations were noted during the observation period.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification: Glyphosate Technical
Description: Solid; molecular weight 169.1 g/mol
Lot/Batch #: 20070606

Purity:	98.05 %
Water solubility:	Yes
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control: Not specified

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	17 weeks
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	2.907 – 3.145 kg
Acclimation period:	5 to 6 days
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (Guabi, Mogiana Alimentos S.A. - Brazil), *ad libitum*
Water:	Filtered tap water, *ad libitum*
Housing:	The animals were housed individually in galvanized steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.
Environmental conditions:	Temperature: 17 – 22 °C
	Humidity: 30 – 70 %
	Air changes: 10 – 15 / hour
	Photocycle: 12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The fur from the back was clipped using a small animal clipper (Oster model Golden A5, Electric Razor), 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin (approximately 6 cm^2) and the patch was held in the test site by an adhesive and non-irritating tape (Micropore®).
Chemical preparation:	Glyphosate technical was applied onto a moistened gauze dressing
Chemical application:	0.5 g / animal
Chemical removal:	Skin was washed with physiological saline.

B: Study design and methods

In life dates: 2008-05-20 to 2008-05-24

Animal assignment and treatment:

Each animal provisionally selected for the test was prepared by clipping the fur from the back approximately 24-hours prior to the application of the test item, using a small animal clipper (Oster model Golden A5, Electric Razor) with great care taken to avoid abrading the skin during the clipping procedure, so as not to alter its permeability. The clipped area was large enough to allow clear visualization of the test site. After being clipped, visual examination of the skin confirmed the skin was intact and healthy.

Five-tenths of a gram (0.5 g) of the test item was applied over the skin of each animal. The test item was first placed onto a moistened gauze dressing, which was applied over a small section of the test area (approximately 6 cm^2) in such a manner that there was good contact and uniform distribution of the test item on the skin. After application, the gauze was held in the test site by an adhesive and non-irritating tape. Removal and ingestion of

the test item was prevented by placing a suitable adhesive tape (semi-occlusive dressing) around the trunk and test area. Adjacent untreated shaved areas of the skin were used as the negative control. After the 4-hour exposure period, the gauze patches were removed, any residual test item washed using physiological saline.

The treated areas examined for signs of irritation 1, 24, 48 and 72-hours after removal of the patch according to the table below. During the observation period animals were observed once daily for general health/morbidity and mortality.

The test was performed initially using one single animal for evaluation of any irritant / corrosive effect of the test item to the skin. Because no severe dermal reaction was observed in the initial test, two additional animals were tested to confirm the response.

Skin reaction grading according to Draize criteria used by [redacted] (2008)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef 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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

Body weight was unaffected by the administration of the test item.

D. NECROPSY

No data on necropsy were reported.

E. SKIN OBSERVATIONS

No signs of dermal irritation were observed at any of the time points in any of the animals tested.

Table 6.2.4.4-1 Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted] 2008): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	97 (♀)	0	0
	98 (♀)	0	0

	99 (♀)	0	0
24-hours	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
48 hours	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
72-hours	97 (♀)	0	0
	98 (♀)	0	0
	99 (♀)	0	0
Individual 24 – 72 h means	**97 (♀)**	**0.0**	**0.0**
	98 (♀)	**0.0**	**0.0**
	99 (♀)	**0.0**	**0.0**

C. BODY WEIGHT

All animals presented gain in body weight during the observation period.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for 4 hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for each animal.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.7. Study 7

Data point:	CA 5.2.4/007
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Report No	B02777
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), US EPA OPPTS 870.2500 (1998), 2004/73/EC B.4 (2004), 12 NohSan No. 8147 (2000)
Deviations from current test guideline (OECD 404, 2015)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered acceptable

In a primary dermal irritation study, young adult New Zealand White rabbits (one male and two females) were dermally exposed to glyphosate technical material (Batch: 0507, Purity: 96.1 % w/w glyphosate acid). The clipped, intact skin of the left flank was exposed to 0.5 g of the test material, moistened with about 0.5 mL water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. No clinical signs were observed.

Based on the study, glyphosate technical material does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical Material
Description:	White powder
Lot/Batch number:	0507
Purity:	96.1 % w/w Glyphosate acid
Stability of test compound:	Stable under storage conditions of room temperature (range of 20 °C ± 5 °C), protected from light and humidity. Expiry date: 2008-08

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White (SPF)
Source:	[redacted]
Age:	Male: 10 – 11 weeks; female: 15 – 16 weeks
Sex:	Male and female
Weight at dosing:	Male: 2.440 kg; female: 2.749 and 2.815 kg
Acclimation period:	5 – 6 days
Diet:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba, CH-4303 Kaiseraugst, Switzerland), *ad libitum;* wood blocks (RCC Ltd, Fullinsdorf, Switzerland) and hay sticks 4642 (Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) were provided for gnawing.
Water:	Community tap water, *ad libitum*
Housing:	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photocycle: 12 hours light / dark

4. Test conditions:

Patch site preparation technique:	The left flank was clipped (area of approximately 100 cm^2) with an electric clipper 4 days before treatment.

Patching technique:	Dermal application onto shaved, intact dorsal skin (patch area 2.5 cm × 2.5 cm). The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape.
Chemical preparation:	Glyphosate technical material was moistened with approximately 0.5 mL purified water.
Chemical application:	0.5 g / animal
Chemical removal:	Skin was flushed with lukewarm tap water

B: Study design and methods

In-life dates: 2006-12-18 to 2006-12-22

Animal assignment and treatment:

Three young adult (one male and two female) New Zealand White rabbits were used in the study. As it was suspected that the test substance might produce irritancy, a single animal (one female) was treated first. As no corrosive effect was observed after the 4-hour exposure, the test was completed using the two remaining animals for an exposure period of 4 hours.

Four days before treatment, the left flank was clipped, exposing an area of approximately 100 cm^2 (10 cm × 10 cm). The skin of the animals was examined one day before treatment, and regrown fur of all animals was clipped again. Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5 g of glyphosate technical material was placed on a surgical gauze patch (2.5 cm × 2.5 cm). This gauze patch was applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape. The duration of treatment was 4 hours after which the dressing was removed, and the skin was flushed with lukewarm tap water to clean the application site so that any reactions (erythema) were clearly visible at that time.

Observations for viability, mortality and clinical signs were carried out daily from acclimatisation of the animals to the termination of the study. Body weights of individual animals were recorded at the start of the acclimatisation period, on the day of application, and at termination of the observation period. Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC (see table below) approximately 1, 24, 48 and 72-hours after removal of the dressing, gauze patch and test substance.

Skin reaction grading according to Draize criteria used by [redacted] (2007)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) or eschar formation (injuries in depth preventing erythema) reading	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 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animals during the study.

C. BODY WEIGHT

The body weights of the rabbits were considered to be within the normal range of variability.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

The test substance did not elicit any skin reactions at the application site of any of the three animals at any of the observation times (all scores 0). The individual mean score for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema for each of the animals. No staining of the treated skin was observed.

Table 6.2.4.5-1 Glyphosate Technical Material: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) [redacted] 2007): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
24-hours	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
48 hours	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
72-hours	31 (♂)	0	0
	32 (♀)	0	0
	33 (♀)	0	0
Individual 24 – 72 h means	**31 (♂)**	**0.0**	**0.0**
	32 (♀)	**0.0**	**0.0**
	33 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for 4 hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema

in rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.8. Study 8

Data point:	CA 5.2.4/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)
Report No	B02294
Document No	Not reported
Guidelines followed in study	OECD 404 (2002), Commission Directive 2004/73/EC B.4 (2004), JMAFF 12 NohSan No. 8147 guideline 2-1-4 (2005)
Deviations from current test guideline (OECD 404, 2015)	Yes, the test patch used had a surface of 16 cm^2 instead of 6 cm^2. This deviation is not considered to affect the study outcome.
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

In a primary dermal irritation study, young adult New Zealand albino rabbits (1 male, 2 females) were dermally exposed to glyphosate technical (NUP 05068, Batch: 200609062, Purity: 95.1 %). The clipped, intact skin of the left flank was exposed to 0.5 g of the solid test item, moistened with about 0.5 mL water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.
No skin reactions were observed at the application site of any animal at any observation time point. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Water solubility:	Yes
Stability of test compound:	Stable under storage conditions (20 ± 5 °C) Expiry date: 2008-09-14

2. **Vehicle and/or positive control:** Purified water
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	13 weeks (male); 14 weeks (females)
Sex:	Male and female
Weight at dosing:	Male: 2.662 kg; female: 2.637 kg and 2.970 kg
Acclimation period:	At least four days
Diet/Food:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blocks and hay sticks were provided for gnawing.
Environmental conditions:	Temperature: 17 – 23 °C
	Humidity: 30 – 70 %
	Air changes: 10 – 15 / hour
	Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The left flank was clipped with an electric clipper (approximately 100 cm^2; 10 cm × 10 cm), 4 days before application
Patching technique:	Dermal application onto shaved, intact skin (4 cm × 4 cm). The patch was covered with a semi-occlusive dressing which was wrapped around the abdomen and anchored with tape.
Chemical preparation:	0.5 g glyphosate / animal was weighed and moistened with approximately 0.5 mL of purified water before application.
Chemical application:	0.5 g glyphosate technical
Chemical removal:	Skin was flushed with lukewarm tap water.

B: Study design and methods

In life dates: 2007-01-09 to 2007-01-15

Animal assignment and treatment:

The test was conducted using young adult New Zealand White rabbits (one male, two females). The test was performed in a sequential manner, first using one animal. Since no signs of corrosion were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.5 g of the solid test substance moistened with approximately 0.5 mL of purified water was applied to the intact skin of the clipped left flank of the rabbits on an approx. 16 cm^2 gauze patch. The patch was covered with a semi-occlusive dressing. After 4 hours of exposure the dressing was removed, and the skin was cleaned with lukewarm tap water.

Skin reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72-hours after removal of the patch (see table below). The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Skin reaction grading according to Draize criteria used by [redacted] (2007d)

Skin Reaction	**Grading**
Erythema and eschar formation	

No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation (preventing grading of erythema) reading	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 (raised approx. 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The test substance produced no staining on the treated skin. In addition, neither alterations of the treated skin, nor corrosive effects were observed.

Table 6.2.4.6-1 Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) ([redacted] 2007): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
24-hours	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
48 hours	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
72-hours	7 (♂)	0	0
	8 (♀)	0	0
	9 (♀)	0	0
Individual 24 – 72 h	7 (♂)	**0.0**	**0.0**

means	8 (♀)	**0.0**	**0.0**
	9 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the minor deviation of a test patch size of 16 cm² instead of 6 cm² the study is in concordance with the current OECD TG 404 (2015). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.9. Study 9

Data point:	CA 5.2.4/009
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits
Report No	15278
Document No	P326
Guidelines followed in study	US EPA OPPTS 870.2500 (1998), OECD 404 (2002), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 404, 2015)	Yes, step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Individual animal weights at start and conclusion of the test not provided. No data about the relative humidity, air changes, specific age of animals, or animal body weight were given. The stability of the test chemical is not reported. These deviations are not considered to affect the study outcome.
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

A primary skin irritation test was conducted with three young New Zealand albino rabbits (males) to determine the potential for glyphosate acid technical (Batch: 040205, Purity: 97.23 %) to produce irritation after a single topical application. Five-tenths of a gram (0.5 g) of the test substance was moistened with distilled water and applied to the clipped intact dorsal area and the trunk (6 cm^2) of three rabbits for 4 hours under semi-occlusive conditions. The patch was held in contact with the skin with a Micropore tape. After removal of the patch, the test sites were gently cleansed of any residual test substance. Skin reactions were scored according to the Draize method and recorded 1, 24, 48 and 72-hours after removal of the test material.

One hour after patch removal, one animal showed very slight erythema (score 1). Dermal irritation cleared from this animal by 24-hours. No skin irritation was noted for the other two animals.

Based on the study, glyphosate acid technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Acid Technical (CAS No. 1071-83-6)
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Water solubility:	12 g/L; insoluble in organic solvents
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand albino
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male
Weight at dosing:	Not specified
Acclimation period:	21 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326
Water:	Filtered tap water, *ad libitum*
Housing:	Individual housing in suspended stainless steel cages with mesh floors which conform to the size recommendations in the at study timepoint valid *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.
Environmental conditions:	Temperature: 18 – 22 °C
	Humidity: Not specified
	Air changes: Not specified
	Photoperiod: 12-hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	Clipping the dorsal area and the trunk, 24-hours before application.
Patching technique:	Dermal application onto shaved, intact dorsal skin (6 cm^2) and the patch and entire trunk were wrapped with semi-occlusive 3-inch Micropore tape.
Chemical preparation:	Glyphosate Acid Technical was moistened with distilled water (70 % w/w mixture).
Chemical application:	0.5 g (0.71 g test mixture) / animal
Chemical removal:	Skin was cleaned

B: Study design and methods

In life dates: 2004-05-05 to 2004-05-08

Animal assignment and treatment:
On the day before application, a group of animals was prepared by clipping (Oster model #A5-small) the dorsal area and the trunk. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy animals without pre-existing skin irritation were selected for test. Prior to application, the test substance was moistened with distilled water to achieve a dry paste by preparing a 70 % w/w mixture. Five-tenths of a gram (0.5 g) of the test substance (0.71 g of the test mixture) was placed on an l-inch x l-inch, 4-ply gauze pad and applied to one 6 cm² intact dose site on each animal. The pad and entire trunk of each animal were then wrapped with semi-occlusive 3-inch Micropore tape to avoid dislocation of the pad. Elizabethan collars were placed on each rabbit and they were returned to their designated cages.

After 4 hours of exposure to the test substance, the pads and collars were removed, and the test sites were gently cleansed of any residual test substance. Individual dose sites were scored according to the Draize scoring system at approximately 1, 24, 48 and 72-hours after patch removal. The animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period.

Skin reaction grading according to Draize criteria used by [redacted] (2005d)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef 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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No signs of gross toxicity, adverse effects, or abnormal behaviour were noted.

C. BODY WEIGHT

Body weight was not recorded.

D. NECROPSY

No gross abnormalities were observed at necropsy.

E. SKIN OBSERVATIONS

Two of three sites were free from irritation throughout the study. One hour after patch removal, one animal exhibited very slight erythema. Dermal irritation cleared from this animal by 24-hours. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.7-1 Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits [redacted] 2005): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	11807 (♂)	0	0
	11808 (♂)	1	0
	11809 (♂)	0	0
24-hours	11807 (♂)	0	0
	11808 (♂)	0	0
	11809 (♂)	0	0
48 hours	11807 (♂)	0	0
	11808 (♂)	0	0
	11809 (♂)	0	0
72-hours	11807 (♂)	0	0
	11808 (♂)	0	0
	11809 (♂)	0	0
Individual 24 – 72 h means	**11807 (♂)**	**0.0**	**0.0**
	11808 (♂)	**0.0**	**0.0**
	11809 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, no report of body weights, water solubility and relative humidity are noted. These deviations are not considered to affect the study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours provoke very slight erythema in one of three animals one hour after patch removal, which was cleared within 24-hours. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.10. Study 10

Data point:	CA 5.2.4/010
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Skin Irritation to the Rabbit
Report No	[redacted] P/4695
Document No	Not reported
Guidelines followed in study	OECD 404 (1992), 92/69/EEC B.4 (1992), US EPA 81-5
Deviations from current test guideline (OECD 404, 2015)	No step-wise approach, use of six instead of three animals simultaneously, the temperature was 17 ± 2 °C, no specific age of animals reported and occlusive dressing instead of semi-occlusive dressing. However, these deviations are considered to represent a worst-case scenario, not compromising the negative study outcome.

Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered acceptable

In a primary dermal irritation study, six young adult, New Zealand White, female rabbits were dermally exposed to approximately 0.5 g of glyphosate acid (Batch: P24, Purity: 95.6 %), moistened with 0.5 mL deionised water, for 4 hours to an area (approximately 2.5 cm × 2.5 cm) on the left shorn flank, under an occlusive dressing. The patch was secured by two strips of surgical tape and the exposed skin was covered by impermeable rubber sheeting wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape. After removal of the patch, the application site was gently cleansed with warm water. Skin irritation was scored using the Draize scheme 0.5 – 1, 24, 48 and 72-hours after removal of the test substance.

Under the test conditions chosen, none of the animals showed any test item-related changes. The mean individual score for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate acid does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate acid
Description:	White, solid
Lot/Batch #:	P24
Purity:	95.6 % w/w
Stability of test compound:	The test substance was used within the expiry date (no date provided)

2. **Vehicle and/or positive control:** Deionised water

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White albino
Source:	Animals 1-5: [redacted] Animal 9: [redacted]
Age:	Young adults (not further specified)
Sex:	Female
Weight at dosing:	3001 – 4386 g
Acclimation period:	At least 6 days
Housing:	Individually, in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study
Diet:	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	Temperature: 17 ± 2 °C Humidity: 40 – 70 % Air changes: Approximately 25 / hour Photocycle: 12 hours light / dark cycle

4. **Test conditions:**

Patch site preparation technique:	Approximately 24-hours before application, an area approximately 7 cm x 13 cm on the left flank of each rabbit was clipped.
Patching technique:	Dermal application onto shaved, intact dorsal skin (approximate size 2.5 cm × 2.5 cm). The gauze was secured by two strips of surgical tape which was covered by a piece of impermeable rubber sheeting (approximate size 35 cm × 13 cm) wrapped around the trunk and is secured with adhesive impermeable polyethylene tape (7.5 cm wide).
Chemical preparation:	Approximately 0.5 g glyphosate was moistened with 0.5 mL of deionised water
Chemical application:	0.5 g / animal
Chemical removal:	Skin was gently cleaned using clean swabs of absorbent cotton wool soaked in clean warm water.

B: Study design and methods

In-life dates: 1995-03-09 to 1995-04-28

Animal assignment and treatment:

Approximately one day before treatment, the left flank was clipped with an electric clipper, exposing an area of approximately 7 cm × 13 cm. On the day of treatment, 0.5 g of glyphosate acid (95.6 % w/w) (moistened with approximately 0.5 mL deionised water) was applied to the test site (approximately 2.5 cm × 2.5 cm) on the left flank of six female rabbits. The treated area was covered with a piece of 8-ply surgical gauze (approximate size 2.5 cm × 2.5 cm), which was secured by two strips of surgical tape (approximate size 1 cm × 8 cm). This was covered by a piece of impermeable rubber sheeting (approximate size 35 cm × 13 cm) wrapped once around the trunk of the animal and secured with adhesive impermeable polyethylene tape (7.5 cm wide).

The dressings were left in position for approximately 4 hours. The application site was gently cleansed free of any residual test substance using clean swabs of absorbent cotton wool soaked in clean warm water and was then dried gently with clean tissue paper.

The Draize scale (see table below) was used to assess the degree of erythema and oedema at the application sites approximately 30 – 60 minutes, 1, 2 and 3 days after removal of the dressings. Any other signs of skin irritation were also recorded.

Skin reaction grading according to Draize criteria used by [redacted] (1996)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness)	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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0). The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.8-1 Glyphosate Acid: Skin Irritation Study in Rabbits [redacted] 1996): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
30 – 60 min	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
24-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
48 hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
72-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	9 (♀)	0	0
Individual 24 – 72 h means	**1 (♀)**	**0.0**	**0.0**
	2 (♀)	**0.0**	**0.0**
	3 (♀)	**0.0**	**0.0**
	4 (♀)	**0.0**	**0.0**
	5 (♀)	**0.0**	**0.0**
	9 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of six instead of three animals, occlusive instead of semi-occlusive dressing are noted.
Nevertheless, as the occlusive application is considered to represent a worst-case scenario, this deviation and all other deviations, considered as minor, do not compromise the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
The occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable even though an occlusive dressing was used, because this is considered worst-case and no skin irritation was reported. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in female rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.11. Study 11

Data point:	CA 5.2.4/011
Report author	[redacted]
Report year	1995
Report title	HR-001: Primary Dermal Irritation Study in Rabbits
Report No	[redacted]95-0035
Document No	Not reported
Guidelines followed in study	OECD 404 (1992), US EPA FIFRA Guideline Subdivision F (1984), JMAFF 59 NohSan 4200 (1985)
Deviations from current test guideline (OECD 404, 2015)	No step-wise approach. Six instead of three animals were used simultaneously. It is not mentioned that only intact skin is used for treatment. No clinical signs were reported. Occlusive instead of semi-occlusive dressing. However, this is considered to represent a worst-case scenario, not compromising the negative study outcome. The stability of the test chemical is not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered acceptable

In a primary dermal irritation study, young adult New Zealand albino rabbits (six females) were dermally exposed to glyphosate technical (HR-001, Batch: T-941209, Purity: 97.56 %). The clipped dorso-lumbar region was exposed to 0.5 g of the solid test item, moistened with about 0.5 mL water, for 4 hours under occlusive conditions. The rabbits were observed for 72-hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72-hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	HR-001 (glyphosate technical)
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Water solubility:	12 g/L (25 °C)
Stability of test compound:	Not reported

2. **Vehicle and/or positive control:** Deionised water

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White, Kbl:SPF
Source:	[redacted]
Age:	12 weeks
Sex:	Female
Weight at dosing:	2408 – 2686 g
Acclimation period:	18 days
Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo)
Water:	Water filtrated and sterilized, *ad libitum*
Housing:	Individually in aluminium cages with wire-mesh floor
Environmental conditions:	Temperature: 23.9 – 24.0 °C Humidity: 52.8 – 56.6 % Air changes: 15 / hour Photoperiod: 12 hours light / dark cycle

4. **Test conditions:**

Patch site preparation technique:	Dorsal fur of the trunk was removed with an electric clipper and a shaver approximately 24-hours before application (2.54 cm^2 × 2.54 cm^2, approximately 6 cm^2).
Patching technique:	Dermal application onto shaved, intact dorsal skin. The patch was held in position with a polyethylene sheet and non-irritating adhesive for exposure period duration.
Chemical preparation:	Glyphosate technical was finely ground in a mortar shortly before application.
Chemical application:	0.5 g in 0.5 mL deionised water
Chemical removal:	Washed off with deionised water.

B: Study design and methods

In life dates: 1995-05-16 to 1995-05-19

Animal assignment and treatment:

Glyphosate technical (HR-001) (0.5 g) moistened with 0.5 mL of deionised water was applied to the closely-clipped dorso-lumbar region (6 cm^2) of six female New Zealand rabbits and covered by a semi-occlusive gauze patch for 4 hours. The patch was held in place in an occlusive manner with a polyethylene sheet and non-irritating adhesive tape. At the end of the exposure period, the patch was removed, and the treatment site was washed with distilled water to remove any residual test substance. Thereafter, all animals were observed for primary dermal irritation 1, 24, 48 and 72-hours. Degree of erythema and oedema relative to treatment were recorded during a subsequent 72-hour observation period according to the criteria described in the Guideline of MAFF in Japan and the method of Draize. Body weights were measured prior to application, and after the final observation 72-hours after application.

Skin reaction grading according to Draize criteria used by [redacted] (1995)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef 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 (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Clinical signs were not observed.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No signs of erythema, eschar, oedema and any other evidence of irritation were observed in either the test substance treated site or the negative control site at any time during the observation period. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. The observation period was therefore completed after 72-hours.

Table 6.2.4.9-1 HR-001: Primary Dermal Irritation Study in Rabbits [redacted] 1995): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema and eschar formation	Oedema
1 hour	1 (♀)	0	0
	2 (♀)	0	0

Table 6.2.4.9-1 HR-001: Primary Dermal Irritation Study in Rabbits [redacted] 1995): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema and eschar formation	Oedema
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
24-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
48 hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
72-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♀)	0	0
	5 (♀)	0	0
	6 (♀)	0	0
Individual 24 – 72 h means	**1 (♀)**	**0.0**	**0.0**
	2 (♀)	**0.0**	**0.0**
	3 (♀)	**0.0**	**0.0**
	4 (♀)	**0.0**	**0.0**
	5 (♀)	**0.0**	**0.0**
	6 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of six instead of three animals, occlusive instead of semi-occlusive dressing and no report of clinical signs are noted.
Nevertheless, as the occlusive application is considered to represent a worst-case scenario, this deviation as well as all other deviations is not compromising the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable even though an occlusive dressing was used and clinical signs were not reported, because this is considered worst-case and no skin irritation was reported. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in female rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.12. Study 12

Data point:	CA 5.2.4/012

Report author	[redacted]
Report year	1994
Report title	Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit
Report No	545/40
Document No	Not reported
Guidelines followed in study	US EPA 81-5 (1984), US EPA 798.4470
Deviations from current test guideline	Yes, step-wise approach by initial testing in one animal was not performed and animals were treated simultaneously. Six instead of three animals were used. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Water solubility and other physicochemical properties (with the exception of vapour pressure) were not documented.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive (low purity: 46.1 % glyphosate), Category 3a **Conclusion AGG:** The study is considered acceptable

In an acute dermal irritation study, one male and five female New Zealand White rabbits were dermally exposed to glyphosate premix (Batch: 290-JaK-146-4; Purity: 46.1 % glyphosate). The fur was clipped from the dorsal / flank area of each rabbit on the day before treatment. A quantity of 0.5 mL of test material was applied to the intact skin of each rabbit under a 2.5 cm × 2.5 cm gauze patch for 4 hours under semi-occlusive conditions. Following removal of the patch the skin was gently swabbed with cotton wool soaked in distilled water to remove residual test material. The rabbits were observed for 72-hours. Skin reactions were evaluated 1, 24, 48 and 72-hours after patch removal and scored according to the Draize classification scheme.

Very slight erythema was noted in two animals 1 hour after patch removal. Very slight erythema persisted in one animal at the 24-hour observation time point and was also noted in one other animal. No other skin reactions were noted for any animal at any other observation time point. The mean score for the 24, 48 and 72-hour readings was 0.33 for erythema for two animals, 0.0 for erythema for four animals and 0.0 for oedema of all animals.

Based on the study, glyphosate premix does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Premix (aqueous solution containing isopropylamine salt of glyphosate as the active ingredient)
Description:	Pale yellow liquid
Lot/Batch #:	290-JaK-146-4
Purity:	62.2 % as glyphosate isopropylamine salt (46.1 % as glyphosate)
Water solubility:	Not specified
Stability of test compound:	Expiry date: 1995-09-30

2. **Vehicle and/or positive control:** None
3. **Test animals:**

Species:	Rabbit

Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 12 – 20 weeks old
Sex:	Male and female
Weight at start of dosing:	2.19 – 2.52 kg
Acclimation period:	At least 5 days
Diet/Food:	STANRAB SQC Rabbit Diet (Special Diet Services Ltd., Witham, Essex, UK) *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended metal cages
Environmental conditions:	Temperature: 18 – 22 °C
	Humidity: 46 – 59 %
	Air changes: Approximately 15 changes per hour
	Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal / flank area of each rabbit was clipped (area not specified), 24 h before application.
Patching technique:	Dermal application onto clipped, intact skin (2.5 cm × 2.5 cm gauze patch). The gauze patch was secured with surgical adhesive tape (BLENDERM) and wrapped in elasticated corset (TUBIGRIP) (semi-occlusive dressing).
Chemical preparation:	0.5 mL of test material was introduced under the 2.5 × 2.5 cm gauze patch.
Chemical application:	0.5 mL glyphosate premix
Chemical removal:	Gentle swabbing with cotton wool soaked in distilled water

B: Study design and methods

In life dates: 1994-03-29 to 1994-04-01

Animal assignment and treatment:
On the day before the test, each of a group of six New Zealand White rabbits (one male and five female) was clipped free of fur from the dorsal/flank area. Only animals with a healthy intact epidermis by gross observation were selected for the study. An amount of 0.5 mL of the test material was introduced to the intact skin of each rabbit under a 2.5 cm × 2.5 cm gauze patch for 4 hours under semi-occlusive conditions. The patch was secured with surgical adhesive tape (BLENDERM), and the trunk of each animal wrapped in an elasticated corset (TUBIGRIP). After 4 hours of exposure the patch was removed, and the skin was gently swabbed with cotton wool soaked in distilled water to remove residual test material.

Test sites were examined for evidence of primary skin irritation 1, 24, 48 and 72-hours after removal of the patch. Skin reactions were scored according to the Draize scale (see table below). Any other skin reactions, if present, were also recorded.

Skin reaction grading according to Draize criteria used by [redacted] (1994)

Skin Reaction	**Grading**
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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were recorded.

C. BODY WEIGHT

Body weight and body weight gain was not recorded.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited very slight erythema in two animals 1 hour after patch removal, which persisted in one animal at the 24-hour observation time point, and was also noted in one other animal at this time point. No other adverse skin reactions were noted for any other animal at any observation time point. The mean score for the 24, 48 and 72-hour readings was 0.33 for erythema for two animals, 0.0 for erythema for four animals and 0.0 for oedema of all animals. Neither alterations of the treated skin, nor corrosive effects were observed.

Table 6.2.4.10-1 Glyphosate Premix: Acute Dermal Irritation Test in the Rabbit [redacted] 1994): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	63 (♂)	1	0
	65 (♀)	0	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	1	0
	100 (♀)	0	0
24-hours	63 (♂)	1	0
	65 (♀)	1	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	0	0
	100 (♀)	0	0
48 hours	63 (♂)	0	0
	65 (♀)	0	0

	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	0	0
	100 (♀)	0	0
72-hours	63 (♂)	0	0
	65 (♀)	0	0
	75 (♀)	0	0
	87 (♀)	0	0
	105 (♀)	0	0
	100 (♀)	0	0
Individual 24 – 72 h means	**63 (♂)**	**0.33**	**0.0**
	65 (♀)	**0.33**	**0.0**
	75 (♀)	**0.0**	**0.0**
	87 (♀)	**0.0**	**0.0**
	105 (♀)	**0.0**	**0.0**
	100 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is with the exception of some minor deviations (no step-wise approach, 6 instead of 3 animals, water solubility and other physicochemical properties not provided) in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid. Nevertheless, due to the low purity of the test substance (46.1 % as glyphosate), the reliability of the study is considered supportive, only.
A single semi-occlusive application of glyphosate premix to intact rabbit skin for four hours elicited very slight erythema at the application site of two animals at the observation time of 24-hours. No skin reactions were noted with the remaining four animals at any observation time.
Individual mean scores over 24, 48 and 72-hours were 0.33 for two animals and 0.0 for four animals for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered acceptable. Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.11 for erythema and 0.0 for oedema in rabbits.

B.6.2.4.13. Study 13

Data point:	CA 5.2.4/013
Report author	[redacted]
Report year	1994
Report title	Glyphosate 360 g/L: Acute dermal irritation test in the rabbit.
Report No	710/29
Document No	Not reported
Guidelines followed in study	OECD 404 (1992)
Deviations from current test guideline	Purity of the test substance not reported. The study report does not include data on mortality, clinical signs, bodyweight or necropsy.
GLP	Yes
Previous evaluation	Not accepted in RAR (2015)
Acceptability/Reliability	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b* **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written below was prepared by the AGG. The study is considered acceptable but with restrictions (reliable

	with restrictions) since the purity of the test substance was not reported.

Short description of study design and observations:	In an acute dermal irritation study, three (two male, one female) New Zealand White rabbits were dermally exposed to 0.5 mL of the test substance (glyphosate salt, batch 1056, purity 360 g/L formulation containing IPA). The fur was clipped free of fur from the dorsal flank area using veterinary clippers. Only animals with a healthy intact epidermis by gross observation were selected for the study. The test material was placed on the shorn skin under a 2.5 cm x 2.5 cm cotton gauze patch which was secured in position with a strip of surgical adhesive tape. To prevent the animals interfering with the patches, the trunk of each rabbit was wrapped in an elasticated corset and the animals were returned to their cages for the duration of the exposure period. Four hours after application the corset and patches were removed from each animal and any residual test material removed by gentle swabbing with cotton wool soaked in distilled water. Approximately one hour following the removal of the patches, and 24, 48 and 72 hours later, the test sites were examined for evidence of primary irritation and scored according to the Draize scale.
Short description of results:	Very slight erythema was noted at one test site at the 1-hour observation. All animals had recovered by the 24 hour observation. The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) since the purity of the test substance, data on mortality, clinical signs, bodyweight and necropsy are not reported. Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits.

B.6.2.4.14. Study 14

Data point:	CA 5.2.4/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate ([redacted]): Primary Dermal Irritation Study in Rabbits
Report No	[redacted] 93-404/N
Document No	Not reported
Guidelines followed in study	OECD 404 (1981)
Deviations from current test guideline (OECD 404, 2015)	No information if a vehicle was used. According to OECD 404 (2015) solid test chemicals should be moistened sufficiently to ensure good skin contact. Occlusive instead of semi-occlusive dressing was used. Step-wise approach by initial testing in one animal was not performed. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. No information was presented on water solubility. The age, source and sex of the animals and weight at dosing was not reported. Four instead of three animals exposed simultaneously under occlusive instead of semi-occlusive conditions. Body weights were not recorded. Abraded skin was included in the test.
Previous evaluation	Not accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive (Sufficient moistening to ensure good skin contact is uncertain), Category 3a **Conclusion AGG:** The study is considered unacceptable since there is no information on moistening of the test item and due to the limited reporting.

In a primary dermal irritation study, four New Zealand White rabbits were dermally exposed to glyphosate (Batch: 36300892; Purity: 99.6 %). The fur was clipped from the back of each rabbit the day prior to dosing. Five tenths (0.5) g of the test item was applied to two intact and two abraded test sites of each rabbit. Test sites were covered with Folpack foil and fixed with Leucoplast for 4 hours. Subsequently, the occlusive dressing was removed and the test sites were washed with warm water and gently dried with a towel.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the binders. Skin reactions were scored according to the Draize method. Animals were observed daily for mortality and overt pharmacotoxic signs.

No skin reactions were observed at the application sites of any animal at any observation time point. The individual mean score for the 24, 48 and 72-hour readings were 0.0 for all animals for erythema and 0.0, 0.0, 0.0 and 0.333 for oedema. No mortality was observed and no clinical signs were noted in any animal.

Thus, glyphosate was not considered a skin irritant.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	99.6 %
Water solubility:	Not specified
Stability of test compound:	Expiry date: 1994-09-01

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Not specified
Age:	Not specified
Sex:	Not specified
Weight at dosing:	Not specified
Acclimation period:	At least 5 days
Diet/Food:	Standard rabbit chow (not further specified) with fresh carrots, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed in wise box

Environmental conditions:	Temperature:	18 ± 2 °C
	Humidity:	40 – 70 %
	Air changes:	10 times / hour
	Photocycle:	12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The back of each rabbit was clipped and distributed into distinct areas (two intact sites and two abraded sites) 24 h before application. Remaining hair was removed with MELT, an intimate depilating product for human use.
Patching technique:	Dermal application onto clipped, non-abraded skin (right-hand / forquarter and left-hand / hindquarter) and abraded skin (right-hand / hindquarter and right-hand / forquarter). Test sites were covered with Folpack fixed with Leucoplast.
Chemical preparation:	Not specified
Chemical application:	Test article (0.5 g) was topically applied to two intact and two abraded dorsal test sites
Chemical removal:	Exposure sites were washed with abundant warm water and dried carefully with a towel

B: Study design and methods

In life dates: 1993-11-29 to 1993-12-02

Animal assignment and treatment:
The test was conducted using four New Zealand White rabbits. Test article (0.5 g) was applied to two intact and two abraded test sites on the back of each of the four animals. Test sites were covered with Folpack foil and fixed with Leucoplast for 4 hours. After exposure the tape and covering were removed and the skin was washed with abundant warm water and gently dried with a towel.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the covering. Skin reactions were scored according to the Draize method. Animals were observed daily for mortality and clinical signs.

Skin reaction grading according to Draize criteria used by [redacted] (1994)

Skin Reaction	**Grading**
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 (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

II. RESULTS AND DISUSSION

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were noted.

C. BODY WEIGHT

Body weight and body weight gain were not recorded.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

Very slight oedema (barely perceptible) was observed at one site in one animal after 24-hours. However, this finding was not observed at any time point thereafter. All other animals did not show any signs of erythema or oedema in response to the application of glyphosate technical for 4 hours. The mean score for the 24, 48 and 72-hour readings were 0.0 for all animals for erythema and 0.0, 0.0, 0.333 and 0.0 for oedema.

Table 6.2.4.14-11 Glyphosate [redacted]): Primary dermal irritation study in rabbits [redacted] 1994): Skin irritation scores for intact skin (individual values)

Evaluation interval	Animal	Erythema (L/R)	Oedema (L/R)
1 hour	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
24-hours	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/1
	4	0/0	0/0
48 hours	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
72-hours	1	0/0	0/0
	2	0/0	0/0
	3	0/0	0/0
	4	0/0	0/0
Individual 24 – 72 h means	1	**0.0**	**0.0**
	2	**0.0**	**0.0**
	3	**0.0**	**0.333**
	4	**0.0**	**0.0**

III. Conclusions

Based on the experimental results the test substance glyphosate is considered as non-irritant to skin.

3. Assessment and conclusion

Assessment and conclusion by applicant:
As the occlusive application is considered to represent a worst-case scenario, this deviation does not compromise the negative study outcome. Nevertheless, as the solid test chemical is not known if moistened and sufficient exposure is uncertain, the study is considered supplementary, only.
A single occlusive application of glyphosate to intact rabbit skin for four hours elicited a very slight reaction in one site at 24-hours; all other sites had no skin reactions at any observation time. The individual mean score over

24, 48 and 72-hours was 0.0 for erythema for all animals and 0.333 for one animal for oedema, and 0.0 for all other animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered unacceptable since there is no information on moistening the test item and due to the limited reporting and the occlusive dressing used. Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.08 for oedema in rabbits.

B.6.2.4.15. Study 15

Data point:	CA 5.2.4/015
Report author	[redacted]
Report year	1991
Report title	Primary Skin Irritation Study with Glyphosate Technical (FSG 03090 H/05 March 90) in New Zealand White Rabbits
Report No	[redacted]878.SKIN
Document No	TOXI-878/1990
Guidelines followed in study	OECD 404 (1987)
Deviations from current test guideline (OECD 404, 2015)	Step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. It is not mentioned that only intact skin is used for treatment. Water solubility and physicochemical properties were not documented. Volume of the vehicle distilled water used to form slurry was not reported. Occlusive (aluminium foil) instead of semi-occlusive dressing. However, this is considered to represent a worst-case scenario, not compromising the negative study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting.

A primary skin irritation test was carried out using two male and one female New Zealand White rabbits to determine the potential of glyphosate technical (Batch: 60; Purity: 96.8 %) to produce dermal irritation after single topical exposure. The test compound (0.5 g mixed with distilled water into a slurry) was applied for four hours to the skin of the rabbits using an aluminium foil patch of 3 cm × 3 cm (occlusive exposure conditions). After removal of the patch, the application area was washed off with distilled water. Skin reactions were scored according to the Draize method and recorded 1, 24, 48 and 72-hours after removal of the test-patch. Animals were observed daily for mortality and clinical signs. Body weights were recorded at the start of acclimatisation, the day of application and at study termination. The study was terminated on day 3, since no cutaneous reaction was present in any animal. Animals were sacrificed and subjected to necropsy.

No mortalities occurred. No signs of systemic toxicity and no noteworthy changes in body weight were noted. There was no skin reactions observed in any animal at any observation time-point. Therefore, the study was terminated after the 72-hour reading time-point. No gross pathological findings were noted at necropsy.

The mean for the 24, 48 and 72-hour readings for each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (FSG 03090 H/05 March 90)
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	60
Purity:	96.8 %
Water solubility:	Not soluble / suspendable in water
Stability of test compound:	Expiry date: 1992-07

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 14 weeks
Sex:	Male and female
Weight at dosing:	1.8 – 2.1 kg
Acclimation period:	At least 4 days
Diet/Food:	Standard "Gold Mohur" pelleted rabbit maintenance diet (M/s Lipton India Ltd., Bangalore 560 052, India), *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
Housing:	Individually housed in stainless steel / aluminium cages
Environmental conditions:	Temperature: 23 ± 2 °C Humidity: 68 ± 6 % Air changes: 10 – 15 air changes per hour Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal fur of each rabbit was clipped (approximate area 10 × 10 cm), 24 h before application.
Patching technique:	Test patch dermally applied to dorsolateral thoracic skin. The patch was secured in position with adhesive tape and wrapped with an elastic bandage around the abdomen.
Chemical preparation:	0.5 g of test compound was finely ground and mixed with distilled water (volume not specified) to form a slurry and spread evenly over a 3 × 3 cm aluminium foil patch.
Chemical application:	Foil patch with slurry (0.5 g of test compound) was topically applied to dorsolateral thoracic skin. Animals were fixed in pyrogen test cages for the 4 hour exposure period.
Chemical removal:	Skin flushed with distilled water

B: Study design and methods

In life dates: 1990-09 (not further specified)

Animal assignment and treatment:

The test was conducted using two male and one female New Zealand White rabbits. Approximately 24 hours before treatment the dorsal fur was clipped with an electric clipper, exposing an area of approximately 100 cm² (10cm x 10 cm). Test compound (0.5 g glyphosate technical mixed with distilled water [amount not specified] into a slurry) was applied to the dorsolateral thoracic skin of each rabbit using an aluminium foil patch (3 × 3 cm). A control patch of bare aluminium foil was applied 3 – 4 cm posterior to the test patch (occlusive). Both patches were secured in position with adhesive tape and wrapped with an elastic bandage around abdomen. Animals were fixed in pyrogen test cages for a 4 hour exposure period. Thereafter, the dressing was removed, and the skin flushed with distilled water.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the dressing. Skin reactions were scored according to the Draize method (see table below). Animals were observed for mortality and clinical signs daily. Body weights were recorded at the start of the acclimatisation period, on the day of application, and at termination. Necropsy was performed in animals sacrificed at termination.

Skin reaction grading according to Draize criteria used by [redacted] (1991d)

Skin Reaction	**Grading**
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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations or symptoms of toxicity were noted.

C. BODY WEIGHT

There were no noteworthy changes in body weight.

D. NECROPSY

No gross abnormalities were observed at necropsy.

E. SKIN OBSERVATIONS

No skin reactions were observed at the application site of any animal at any observation time point (all scores were 0) following 4 hours occlusive exposure to glyphosate technical. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.15-12 Primary Skin Irritation Study with Glyphosate Technical (FSG 03090 H/05 March 90) in New Zealand White Rabbits [redacted] 1991): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	RS 007 (♂)	0	0
	RS 008 (♂)	0	0
	RS 009 (♀)	0	0
24-hours	RS 007 (♂)	0	0
	RS 008 (♂)	0	0
	RS 009 (♀)	0	0
48 hours	RS 007 (♂)	0	0
	RS 008 (♂)	0	0
	RS 009 (♀)	0	0
72-hours	RS 007 (♂)	0	0
	RS 008 (♂)	0	0
	RS 009 (♀)	0	0
Individual 24 – 72 h means	**RS 007 (♂)**	**0.0**	**0.0**
	RS 008 (♂)	**0.0**	**0.0**
	RS 009 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, no report of water solubility, physicochemical properties, volume of the vehicle, occlusive (aluminium foil) instead of semi-occlusive dressing are noted. Nevertheless, as the occlusive application is considered to represent a worst-case scenario, this deviation and all other deviations, considered as minor, do not compromise the negative study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
The occlusive application of glyphosate to rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits.

B.6.2.4.16. Study 16

Data point:	CA 5.2.4/016
Report author	[redacted]
Report year	1991
Report title	Acute dermal irritation study in New Zealand White rabbits treated with the test article glyphosate technico 98 %
Report No	910259
Document No	Not reported
Guidelines followed in study	Not reported
Deviations from current test guideline	Yes, step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. The study report does not include data on bodyweight or

	necropsy.
GLP	No
Previous evaluation	Not accepted in RAR (2015)
Acceptability/Reliability	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b* **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written below was prepared by the AGG. The study is considered unacceptable since it was not performed according to GLP

Short description of study design and observations:	In an acute dermal irritation study, three male New Zealand White rabbits were dermally exposed to 0.5 g test material (glyphosate acid, purity 98 %, moistened with saline). Approximately 24 hours before the test, fur was clipped from the trunk of the animals. Care was taken to not abrade the skin. Only animals with intact skin were used. The test article was applied to a small area (approximately 6 cm²) of skin and covered with a gauze patch which was loosely held in contact with the skin by an impermeable, non-irritant, hypoallergenic tape. Since the test article was a powder, it was applied directly onto the gauze patch after being moistened with 1 ml sterile saline. Four hours after application the patch was removed and the skin reaction was scored according to the Draize scale at 1, 24, 48 and 72 hours after removal.
Short description of results:	No animals died and no clinical signs or behavioral alterations were noted. Slight erythema (graded 1) was observed in all treated rabbits at the 1 and 24 hour observation. **Table 6.2.4.16-1 Skin irritation scores (individual values)**

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1M (♂)	1	0
	2M (♂)	1	0
	3M (♂)	1	0
24-hours	1M (♂)	1	0
	2M (♂)	1	0
	3M (♂)	1	0
48 hours	1M (♂)	0	0
	2M (♂)	0	0
	3M (♂)	0	0
72-hours	1M (♂)	0	0
	2M (♂)	0	0
	3M (♂)	0	0
Individual 24 – 72 h means	1M (♂)	**0.33**	**0.0**
	2M (♂)	**0.33**	**0.0**
	3M (♂)	**0.33**	**0.0**

Assessment and conclusion by RMS:
The study is considered unacceptable since it was not performed according to GLP. Therefore, no conclusion can be drawn. This conclusion is in line with the previous evaluation (RAR, 2015)

B.6.2.4.17. Study 17

Data point:	CA 5.2.4/017
Report author	[redacted]

Report year	1990
Report title	Acute Dermal Irritation/Corrosion of Glyphosate Technical in the Rabbit (Intact and Abraded Skin)
Report No	[redacted] 900822A
Document No	Not reported
Guidelines followed in study	OECD 404 (1981), Method B.4 84/449/EEC
Deviations from current test guideline (OECD 404, 2015)	Yes, step-wise approach by initial testing in one animal was not performed and three animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Volume of saline used as vehicle to moisten test substance was not reported. The water solubility and stability of the test compound were not specified. Justification for use of vehicle and justification for choice of vehicle (if other than water) are missing from study report. Body weights at start and termination of study, age of animals, acclimation period, and environmental conditions were not reported. The study also includes investigations on the abraded skin. These deviations are not considered to affect the study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting

A primary skin irritation test was carried out using three male New Zealand White rabbits to evaluate the potential of glyphosate technical (Batch: 0190 A; Purity: 98.1 %) to produce dermal irritation after single topical exposure on intact and abraded dorsal skin for 4 hours. The test compound (0.5 g moistened with saline) was applied for four hours to the skin of the rabbits using gauze pad (6 cm^2) secured with non-irritating tape (semi-occlusive exposure conditions). Untreated shaved skin and abraded skin sites served as comparator controls for each animal. After removal of the dressing, the application area was washed off with sterile physiological saline. Skin reactions were scored according to the Draize method and recorded 1, 24, 48 and 72-hours after removal of the patch. Animals were observed for mortality and clinical signs. Body weights were recorded at the start of application.

No mortalities occurred. No signs of toxicity were noted. There were no signs of erythema or oedema observed in any animal at any observation time-point on either intact or abraded skin. The mean for the 24, 48 and 72-hour readings for each animal was 0.0 for erythema and 0.0 for oedema, respectively.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen. Thus, glyphosate technical does not warrant classification as being irritating / corrosive to the skin.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Yellowish (transparent)
Lot/Batch #:	0190 A
Purity:	98.1 %
Water solubility:	Not specified

Stability of test compound:	Not specified

2. Vehicle and/or positive control: 0.9 % sterile physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Adult (not further specified)
Sex:	Male
Weight at dosing:	2.2 – 2.4 kg
Acclimation period:	Not specified
Diet/Food:	Standard rabbit diet (Redmills, Goresbridge, Co. Kilkenny, Ireland), *ad libitum*
Water:	Drinking water, *ad libitum*
Housing:	Individually housed in standard grid bottom rabbit cages
Environmental conditions:	Temperature: Not specified
	Humidity: Not specified
	Air changes: Not specified
	Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The dorsal fur of each rabbit was clipped (approximate area 6 × 6 cm), 24 h before application. A sterile 20 gauge Microlance needle was used to abrade the skin prior to application of the test substance.
Patching technique:	Gauze pads (6 cm^2) with test substance were applied directly to the intact and abraded test sites and secured with non-irritating tape (Elastoplast elastic adhesive bandage B.P.).
Chemical preparation:	Test substance (0.5 g) was moistened with saline (volume not specified) and applied to a gauze pad ("Propax" gauze pads).
Chemical application:	Gauze pads (6 cm^2) with 0.5 g of test substance were applied directly to the intact and abraded test sites for 4 hours.
Chemical removal:	Skin washed with sterile physiological saline

B: Study design and methods

In life dates: 1990-03-30 – 1990-08-22 (completion of final report)

Animal assignment and treatment:

The test was conducted using three male adult New Zealand White rabbits. Fur was clipped (approximately 6 × 6 cm) from each animal 24-hours before testing. Skin was abraded using a sterile 20 gauge microlance needle prior to application of test substance. Test compound (0.5 g moistened with saline) applied on a gauze pad (6 cm^2) was directly applied to the intact or abraded skin test sites of each animal. Gauze pads were secured using Elastoplast elastic adhesive bandage. Untreated shaved skin and abraded skin sites served as controls for each animal. After 4 hours of exposure the adhesive dressings were removed, and residual test substance was washed off using sterile physiological saline.

Animals were examined for signs of erythema or oedema 1, 24, 48 and 72-hours after patch removal. Additionally, animals were observed for any other lesions or signs of toxic effects. Irritation responses were scored according to Draize criteria provided in the table below.

Skin reaction grading according to Draize criteria used by [redacted] (1990)

Skin Reaction	Grading
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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations or symptoms of toxicity were noted.

C. BODY WEIGHT

Body weights were not reported.

D. NECROPSY

Necropsy was not performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application or control sites of any animal for any observation time point (all scores were 0). The mean for the 24, 48 and 72-hour readings of each animal were 0.0 for erythema and 0.0 for oedema, respectively.

Table 6.2.4.17-13 Acute Dermal Irritation/Corrosion of Glyphosate Technical in the Rabbit (Intact and Abraded Skin) [redacted], 1990): Skin irritation scores (individual values for intact skin)

Evaluation interval	Animal No.	Erythema*	Oedema*
1 hour	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
24-hours	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
48 hours	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0

72-hours	BL-751 (♂)	0/0	0/0
	BL-761 (♂)	0/0	0/0
	BL-802 (♂)	0/0	0/0
Individual 24 – 72 h means	**BL-751 (♂)**	**0.0**	**0.0**
	BL-761 (♂)	**0.0**	**0.0**
	BL-802 (♂)	**0.0**	**0.0**

*: skin reaction score of treated site (intact skin) / and control site

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in accordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits.

B.6.2.4.18. Study 18

Data point:	CA 5.2.4/018
Report author	[redacted]
Report year	1989
Report title	Glyphosate Technical: Primary Skin Irritation in Rabbits
Report No	5885
Document No	Not reported
Guidelines followed in study	US EPA 81-5
Deviations from current test guideline (OECD 404, 2015)	Yes, step-wise approach by initial testing in one animal was not performed and animals were treated simultaneously. Six instead of three animals used. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. Purity of the test substance not provided. Nevertheless, from the batch number a purity of 98.6 % was concluded. Water solubility and physicochemical properties were not documented. Volume of vehicle for moistening the test material was not recorded. Stability of the test material was not documented. Animal age, clinical signs, necropsy, and body weights were not recorded. Temperature range outside the required range which is not considered to affect the outcome of the study. These deviations are not considered to affect the study outcome.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting

In a primary dermal irritation study, two male and four female nulliparous and non-pregnant young adult New Zealand White rabbits were dermally exposed to glyphosate technical (Batch: 206-Jak-25-1; Purity: 98.6 %). The hair was clipped from the dorsal area of the trunk of each rabbit. The test material (0.5 g moistened with water), was applied to the intact skin of each rabbit under a 2.5 cm × 2.5 cm patch of gauze for 4 hours under semi-occlusive conditions. Following removal of the patch the skin was wiped with a damp tissue to remove surplus test material. The rabbits were observed for 72-hours. Skin reactions were evaluated 1, 24, 48 and 72-hours after patch removal and graded according to the EPA recommended scoring system.

No skin reactions were observed at the application site of any animal at any observation time point. The overall mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Based on the study, glyphosate technical does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	Not specified in the study report Batch 206-JaK-25-1 reported with 98.6 %, see [redacted] 1991.
Water solubility:	Not specified
Stability of test compound:	Not specified

2. **Vehicle and/or positive control:** Water

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adults (not further specified)
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Not specified
Acclimation period:	8 days
Diet/Food:	Standard rabbit diet (Special Diet Services, 1 Stepfield, Witham, Essex, CM8 3AD, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with grid floors and peat moss filled trays beneath
Environmental conditions:	Temperature: 19 – 24 °C (mean minimum and maximum)
	Humidity: 57 % (mean relative humidity)
	Air changes: Not specified
	Photoperiod: 12 hours light / dark cycle

4. **Test conditions:**

Patch site preparation technique:	The dorsal area of the trunk of each rabbit was clipped (area not specified), 24 h before application.
Patching technique:	Dermal application onto clipped, intact skin (2.5 cm × 2.5 cm gauze patch). The patch was covered with Micropore tape and the trunk was loosely bound with Elastoplast Elastic Bandage (semi-occlusive dressing).
Chemical preparation:	0.5 g glyphosate / animal was weighed and moistened with water (amount not specified) before application.
Chemical application:	0.5 g glyphosate technical
Chemical removal:	Skin was wiped with damp tissue

B: Study design and methods

In life dates: 1989-06-14 to 1989-06-18

Animal assignment and treatment:

The hair was clipped from the dorsal area of the trunk of each rabbit approximately 24-hours before treatment. Care was taken to avoid abrading the skin. The test was conducted using two male and four female young adult New Zealand White rabbits. An amount of 0.5 g of the test material (moistened with water) was applied to the intact skin of each rabbit under a 2.5 cm × 2.5 cm patch of gauze under semi-occlusive conditions. After 4 hours of exposure, the patch was removed, and the skin was wiped with damp tissue to remove surplus test material.

Skin reactions were assessed 1, 24, 48 and 72-hours after removal of the patch. Skin reactions were graded according to the EPA Recommended Scoring System (see table below).

Skin reaction grading according to EPA Recommended Scoring System used by [redacted] (1989)

Skin Reaction	**Grading**
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 (area raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were recorded.

C. BODY WEIGHT

Body weight and body weight gain was not recorded.

D. NECROPSY

Necropsy was not performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application site of any animal for any observation time point (all scores were 0) following 4 hours semi-occlusive exposure to glyphosate technical. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.18-14 Glyphosate Technical: Primary Skin Irritation in Rabbits [redacted] 1989): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
1 hour	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
24-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
48 hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
72-hours	1 (♀)	0	0
	2 (♀)	0	0
	3 (♀)	0	0
	4 (♂)	0	0
	5 (♀)	0	0
	6 (♂)	0	0
Individual 24 – 72 h means	**1 (♀)**	**0.0**	**0.0**
	2 (♀)	**0.0**	**0.0**
	3 (♀)	**0.0**	**0.0**
	4 (♂)	**0.0**	**0.0**
	5 (♀)	**0.0**	**0.0**
	6 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations noted were no step-wise approach, use of six instead of three animals, no purity of the test substance provided (concluded from the batch number), no information on water solubility, physicochemical properties, volume of vehicle for moistening the test material, stability of the test material, animal age, and body weights were not recorded, and temperature range outside the required range. Nevertheless, the deviations are not considered to affect the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours

was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits.

B.6.2.4.19. Study 19

Data point:	CA 5.2.4/019
Report author	[redacted]
Report year	1989
Report title	Primary skin irritation study with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46% of N-phosphonomethylglycine acid) in rabbits (4-hour semi-occlusive application on intact and abraded skin)
Report No	238072
Document No	PRO438
Guidelines followed in study	No final conclusion possible.
GLP	No final conclusion possible.
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations:	0.5 mL of the pure test substance (glyphosate salt, purity 62 %) was applied onto the intact skin of 3 female and onto the abraded skin of 3 male albino rabbits.
Short description of results:	Slight irritation (not further specified)
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b*
Reasons why the study report is not available for submission	-
Acceptability/Reliability:	Conclusion GRG: Category 4b Conclusion AGG: study report not available, so no conclusion could be drawn.

Assessment and conclusion by RMS:
The study report is not available to the current RMS. Since there are 23 other skin irritation studies the lack of this particular study is not considered critical.

B.6.2.4.20. Study 20

Data point:	CA 5.2.4/020
Report author	[redacted]
Report year	1988
Report title	Primary Dermal Irritation Study of Glyphosate Batch/lot/nbr no. XLI-55 in New Zealand White Rabbits
Report No	88.2053.010

Document No	[redacted] 88-29
Guidelines followed in study	US EPA 81-5
Deviations from current test guideline (OECD 404, 2015)	The dermal irritation was evaluated at 0.5, 24, 48 and 72-hours instead of 1, 24, 48 and 72-hours. Six instead of three animals were used. Animals were treated simultaneously. Age of animals and number of air changes were not specified. The water solubility and stability of the test compound were not specified. Justification for use of vehicle and justification for choice of vehicle (if other than water) are missing from study report. These deviations are not considered to affect the study outcome.
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

In a primary dermal irritation study, young adult New Zealand White rabbits (three males and three females) were dermally exposed to glyphosate (Batch: XLI-55, Purity: 97.76 %). Each rabbit was administered 0.5 g of the test article (moistened with 0.5 mL of physiological saline) to the clipped back. Each test site was semi-occluded with a one-inch square gauze patch held in place with Micropore® tape. The patches were removed 4 hours following dose administration. Dermal irritation was scored according to the Draize method at 0.5, 24, 48 and 72-hours after patch removal.

Under the test conditions chosen, none of six male rabbits exposed for 4 hours to 0.5 g glyphosate / patch (semi-occlusive conditions) showed any test item-related changes. The overall mean for the 0.5, 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.
Based on the study, glyphosate does not show a skin irritating potential under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate (*N*-phosphonomethylglycine)
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Water solubility:	Not mentioned in the report
Stability of test compound:	Stored at room temperature

2. **Vehicle and/or positive control:** Physiological saline
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	2 – 3 kg

Acclimation period:	At least five days	
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually in wire mesh cages	
Environmental conditions:	Temperature:	20 – 23.9 °C
	Humidity:	40 – 60 %
	Air changes:	Not specified
	Photocycle:	12 hour light / dark cycle

4. Test conditions:

Patch site preparation technique:	The fur on the back was clipped with an electric clipper, 24-hours before application
Patching technique:	Dermal application onto shaved, intact dorsal skin; patch was held on the test site with Micropore®
Chemical preparation:	0.5 g glyphosate was moistened with 0.5 mL of 0.9 % saline
Chemical application:	0.5 g / animal
Chemical removal:	Skin was wiped with gauze

B: Study design and methods

In life dates: 1998-04-11 to 1998-04-14

Animal assignment and treatment:

Six healthy animals (three males and three females) weighing between 2 – 3 kg were selected randomly from the acclimated colony and assigned to the test group. Selection suitability was based on health, weight requirement and of dorsal skin for testing. The fur on the back of each rabbit was clipped with an electric clipper on the day prior to dose administration. The test article (0.5 g moistened with 0.5 mL physiological saline) was applied topically to each of two intact dorsal test sites per rabbit. Immediately after dosing, the test sites were semi-occluded with a one-inch square gauze patch held in place with tape. The animals were collared during the exposure period to prevent removal of the patches. The patches and collars were removed 4-hours after dose administration and the exposure sites gently wiped with gauze to remove as much non-absorbed test article as possible.

Dermal irritation was evaluated at 0.5, 24, 48 and 72-hours after patch removal. Erythema and oedema were scored separately according to the Draize method (see table below). The animals were observed twice daily for mortality at least five hours apart. Body weights were obtained on study day 1 prior to dose administration. At study termination, the animals were euthanised by intra-cardiac injection of sodium pentobarbital and discarded.

Individual animal scores were obtained at each scoring interval by adding the total erythema and eschar formation scores from both application sites to the total oedema formation scores from both sites and dividing by two.

Skin reaction grading according to Draize criteria used by [redacted] (1988)

Skin Reaction	**Grading**
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef 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 (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Not reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

No dermal irritation was noted following test substance application.

Table 6.2.4.20-15 Primary Dermal Irritation Study of Glyphosate [redacted] 1988): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
0.5 hour	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
24-hours	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
48 hours	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0
	1259 (♀)	0	0
72-hours	1167 (♂)	0	0
	1176 (♂)	0	0
	1177 (♂)	0	0
	1264 (♀)	0	0
	1252 (♀)	0	0

	1259 (♀)	0	0
Individual 24 – 72 h means	1167 (♂)	**0.0**	**0.0**
	1176 (♂)	**0.0**	**0.0**
	1177 (♂)	**0.0**	**0.0**
	1264 (♀)	**0.0**	**0.0**
	1252 (♀)	**0.0**	**0.0**
	1259 (♀)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study is in concordance with the current OECD TG 404 (2015). Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single semi-occlusive application of glyphosate to intact rabbit skin for 4 hours elicited no skin reactions at the application site of any animal at any observation time. The individual mean score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. Under the conditions of the study the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in female rabbits. This conclusion is in line with the previous assessment (RAR, 2015)

B.6.2.4.21. Study 21

Data point:	CA 5.2.4/021
Report author	[redacted]
Report year	1987
Report title	Primary Dermal Irritation Study of MON 8750 in New Zealand White Rabbits
Report No	[redacted]86-431/9308A
Document No	Not reported
Guidelines followed in study	US EPA 81-5 (1982)
Deviations from current test guideline	Yes, step-wise approach by initial testing in one animal was not performed. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. No information was presented on water solubility and stability of the test material. Six instead of three animals exposed simultaneously. Body weights were only recorded on day 1 before dosing. Skin reactions were not evaluated at 1 hour. The study report does not contain data on bodyweight, clinical signs, mortality and necropsy.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting

In a primary dermal irritation study, six male young adult New Zealand White rabbits were dermally exposed to MON 8750. The fur was clipped from the back of each rabbit the day prior to dosing. Test article (0.5 g MON

8750 moistened with 0.5 mL saline) was applied to two non-abraded test sites of each rabbit. Test sites were wrapped with semi-occlusive binders consisting of a 1-inch square gauze patch and Micropore tape for 4 hours. Animals were collared throughout the 4-hour exposure period. After 4 hours of exposure the semi-occlusive binders were removed, and the skin was gently wiped with gauze to remove any non-absorbed test article. Skin reactions were assessed 4, 24, 48 and 72-hours after removal of the binders. Skin reactions were scored according to the Draize method. Animals were observed daily for mortality and overt pharmacotoxic signs.

No skin reactions were observed at the application sites of any animal at any observation time point. The mean score for the 4, 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema. No pharmacotoxic signs were noted in any animal.

Thus, MON 8750 is considered to be not irritating to skin.

Materials and methods

A: Materials

1. Test material:

Identification:	MON 8750, [redacted] 86-431
Description:	White powder
Lot/Batch #:	XLG-225 (Assigned [redacted] Identification 86-0616)
Purity:	90.8 %
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: Saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Not specified
Sex:	Male
Weight at dosing:	2 – 3 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US) *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in stainless steel mesh cages
Environmental conditions:	Temperature: 20.0 – 23.9 °C Humidity: 40 – 60 % Air changes: Not specified Photocycle: 12 hours light / dark cycle

4. Test conditions:

Patch site preparation technique:	The back of each rabbit was clipped (area not specified), 24 h before application.
Patching technique:	Dermal application onto clipped, non-abraded skin (two test sites). Test sites were wrapped in semi-occlusive binders consisting of 1 inch square gauze patch and Micropore tape.

Chemical preparation:	Test article was ground with a pestle and mortar before dosing. 0.5 g MON 8750 / animal / test site was weighed and moistened with 0.5 mL physiological saline before application.
Chemical application:	Test article (0.5 g moistened with 0.5 mL saline) was topically applied to two non-abraded dorsal test sites. Animals were collared during the exposure period.
Chemical removal:	Exposure sites gently wiped with gauze to remove non-absorbed test article.

B: Study design and methods

In life dates: 1986-11-06 to 1986-11-09

Animal assignment and treatment:

The test was conducted using six male young adult New Zealand White rabbits. Test article (0.5 g MON 8750 moistened with 0.5 mL saline) was applied to two non-abraded test sites on the dorsal of each rabbit. Test sites were wrapped with semi-occlusive binders consisting of a 1-inch square gauze patch and Micropore tape for 4 hours. Animals were collared throughout the 4-hour exposure period to prevent removal of the patches. After exposure the semi-occlusive binders were removed, and the skin was gently wiped with gauze to remove any non-absorbed test article.

Skin reactions were assessed 4, 24, 48 and 72-hours after removal of the binders. Skin reactions were scored according to the Draize method (see table below). Animals were observed daily for mortality and overt pharmacotoxic signs. Body weights were obtained on study day 1 prior to dose administration.

Skin reaction grading according to Draize criteria used by [redacted] (1987)

Skin Reaction	**Grading**
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 (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical observations were noted.

C. BODY WEIGHT

Body weight and body weight gain were not recorded.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application sites of any animal for any observation time point (all scores were 0) following 4 hours' semi-occlusive exposure to MON 8750. The mean for the 24, 48 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.21-16 Primary Dermal Irritation Study of MON 8750 in New Zealand White Rabbits ([redacted], 1987): Skin irritation scores (individual values)

Evaluation interval	Animal No.	Erythema (L/R)	Oedema (L/R)
4 hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
24-hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
48 hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
72-hours	86-3383 (♂)	0/0	0/0
	86-3384 (♂)	0/0	0/0
	86-3385 (♂)	0/0	0/0
	86-3386 (♂)	0/0	0/0
	86-3387 (♂)	0/0	0/0
	86-3388 (♂)	0/0	0/0
Individual 24 – 72 h means	**86-3383 (♂)**	**0.0**	**0.0**
	86-3384 (♂)	**0.0**	**0.0**
	86-3385 (♂)	**0.0**	**0.0**
	86-3386 (♂)	**0.0**	**0.0**
	86-3387 (♂)	**0.0**	**0.0**
	86-3388 (♂)	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of six instead of three animals, no report of body weight and no 1-hour evaluation of skin irritation are noted.

A single semi-occlusive application of glyphosate to intact rabbit skin for four hours elicited no skin reactions at the application site of any animal at any observation time. The mean individual score over 24, 48 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting.

Under the test conditions the mean cumulative scores at 24, 48 and 72 hours for all animals is 0.0 for erythema and 0.0 for oedema in rabbits.

B.6.2.4.22. Study 22

Data point:	CA 5.2.4/022
Report author	[redacted]
Report year	1983
Report title	Primary skin irritation test on rabbits with glyphosate (tech) of [redacted]
Report No	400060
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 404 (2015))	Step-wise approach by initial testing in one animal not performed. Rationale for *in vivo* testing and consideration of pre-existing data not documented. No information provided on the Batch number, the description of the test substance, CAS number, justification for use of vehicle and justification for choice of vehicle, animal age, in life dates, environmental conditions and adhesive tape used for occlusion. Six instead of three animals were used, 24-hours instead of 4 hours' exposure. Additionally, abraded skin was exposed. Scoring of skin reactions not performed at reading time-point of 48 hours after application. Additionally, systemic effects, like clinical signs of toxicity as well as body weight were not recorded.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	**Conclusion GRG:** Supportive (limited reporting of test conditions, limited scoring time points), Category 3a **Conclusion AGG:** The study is considered unacceptable since the study was not performed according to GLP and the limited reporting

In a primary skin irritation study, three male and three female New Zealand White rabbits were dermally exposed to glyphosate technical. The fur was clipped from both sides of each rabbit the day prior to dosing. Test article (0.5 g moistened with saline) was applied to abraded and intact test site of each rabbit. A gauze patch was applied on top of the test material and hold in place with an adhesive tape. Animals were restrained throughout the 24-hour exposure period. After 24-hours of exposure, the patches and unabsorbed test material were removed. Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

No skin reactions were observed at the application sites of any animal at any observation time point. The mean score for the 24 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Thus, glyphosate technical does not show a skin irritating potential under the test conditions.

Materials and methods

A: Materials

1. **Test material:**

Identification: Glyphosate technical, sample 8.7.83

Description: White amorphous powder

Lot/Batch #:	R&D Sample
Purity:	95 %
Water solubility:	Not specified
Stability of test compound:	Not specified

2. **Vehicle and/or positive control:** Saline
3. **Test animals:**

Species:	Rabbit
Strain:	NWS
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Weight at dosing:	1.5 – 2.5 kg
Acclimation period:	Not specified
Diet/Food:	Lucerne grass, carrots, germinated grams with wheat bran, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in air-conditioned room with regular lighting condition except during exposure; during exposure animals were housed singly in restrain cages
Environmental conditions:	Not specified

4. **Test conditions:**

Patch site preparation technique:	Both sides of each rabbit (back skin) were clipped (area size not specified), 24 h before application. Skin was abraded on one site of the animal (penetration of s*tratum corneum*, but not dermis)
Patching technique:	Dermal application onto clipped, intact and abraded skin (two test sites). Gauze patch was secured over treated area with adhesive tape.
Chemical preparation:	0.5 g glyphosate / animal / test site was moistened with saline before application.
Chemical application:	Test article (0.5 g moistened with saline [amount not specified]) was topically applied to two test sites (intact and abraded). Animals were restrained during the exposure period (24-hours).
Chemical removal:	Patches and unabsorbed test material were removed at 24-hours.

B: Study design and methods

In life dates: Not specified; finalisation date: 1983-10-18

Animal assignment and treatment:

The test was conducted using three male and three female rabbits. The hair on both sides were removed by electric clipper one day before the treatment. Test article (0.5 g moistened with saline) was applied to the test sites of intact and abraded skin (abrasions penetrating the *stratum corneum* but not the dermis) of each rabbit. A gauze patch was placed on the test site and hold in place with an adhesive tape. Animals were restrained throughout the 24-hour exposure period. After 24-hours of exposure the patches and unabsorbed test material were removed.

Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Clinical observations were not reported.

C. BODY WEIGHT

Body weight and body weight gain was not reported.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

No erythema or oedema were observed at the application sites of any animal for any observation time point (all scores were 0) following a 24-hour exposure to glyphosate technical. The individual mean score for 24 and 72-hour readings were 0.0 for erythema and 0.0 for oedema.

Table 6.2.4.22-17 Primary skin irritation test on rabbits with glyphosate (tech) ([redacted], 1983): Skin irritation scores of intact skin (individual values)

Evaluation interval	Animal No.	Erythema	Oedema
24-hours	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
	6	0	0
72-hours	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	0	0
	6	0	0
Individual 24 – 72 h means	**1**	**0.0**	**0.0**
	2	**0.0**	**0.0**
	3	**0.0**	**0.0**
	4	**0.0**	**0.0**
	5	**0.0**	**0.0**
	6	**0.0**	**0.0**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, 6 instead of 3 animals, 24-hours exposure instead of 4 hours, no scoring of skin effects at reading time points of 48 hours after application were noted. No information is provided on the batch number, the description of the test substance, CAS number, animal age, in life dates, environmental conditions, clinical signs of toxicity and body weights.
As the 24-hour application is considered to represent a worst-case scenario, this deviation is not compromising the negative study outcome. Nevertheless, due to the deviations, the study is considered to be used as supplemental information. Therefore, the study is presented here for the sake of completeness, only.

A single semi-occlusive application of glyphosate to intact rabbit skin for 24-hours elicited no skin reactions at the application site of any animal at any observation time. The mean score over 24 and 72-hours was 0.0 for erythema and 0.0 for oedema for all animals.
Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered unacceptable since the study was not performed according to GLP and the study reporting is very limited. Therefore, no conclusion could be drawn.

B.6.2.4.23. Study 23

Data point:	CA 5.2.4/023
Report author	[redacted]
Report year	1981
Report title	Primary skin irritation of MON 0139 to rabbits
Report No	800259
Document No	Not reported
Guidelines followed in study	None; study conducted prior to test guidelines existence
Deviations from current test guideline (OECD 404 (2015))	Yes, step-wise approach by initial testing in one animal was not performed. Rationale for in-vivo testing and consideration of pre-existing data was not documented. No information provided on the environmental conditions. Six instead of three animals exposed simultaneously for 24-hours instead of 4 hours under occlusive instead of semi-occlusive conditions. Additionally, abraded skin was exposed. Scoring of skin reactions was only performed at reading time-points of 24 and 72-hours, not at 48 hours after application. Additionally, body weight was not recorded and no data was provided on clinical signs, mortality and necropsy. No information provided on the acclimation period, diet, water solubility and stability of test compound.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered unacceptable since the study was not performed according to GLP and the limited reporting

In a primary dermal irritation study, three male and three female young adult New Zealand White rabbits were dermally exposed to MON 0139. The fur was clipped from the back of each rabbit prior to dosing. Test article (0.5 mL MON 0139) was applied to two intact test sites and two abraded test sites of each rabbit and covered with gauze patches. Test sites were wrapped with an occlusive dressing consisting of a latex rubber wrap secured by bandaging and elastic tape. After 24-hours of exposure the occlusive patches were removed, and the skin was any non-absorbed test material was wiped from the treated test sites. Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

Erythema was observed on intact skin in one of six animals 24-hours after patch removal. No oedema was observed at any time point. No erythema was observed 72-hours after patch removal. As exposure and examination of abraded skin is not in concordance with the current guideline, results of this part will not be elaborated on.

Due to the lack of scoring data at the 48 hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case).

The calculated mean individual scores for the 24 – 72-hour readings were 0.67 for one animal and 0.0 for the remaining five animals for erythema and 0.0 for oedema.

Thus, MON 0139 is considered non-irritant to skin.

Materials and methods

A: Materials

1. Test material:

Identification:	MON 0139
Description:	Amber liquid
Lot/Batch #:	SSRT-11012
Purity:	Not specified (IPA, 65 %, according to Monograph 2001)
Water solubility:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White (Isf: (NZW))
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and female
Weight at dosing:	1.87 – 2.73 kg
Acclimation period:	Not specified
Diet/Food:	Not specified, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed (not further specified)
Environmental conditions:	Not specified

4. Test conditions:

Patch site preparation technique:	The back of each rabbit was clipped (area not specified) before application. The skin of one of the test sites was abraded, the other one intact. Abrasion was achieved with a hypodermic needle, sufficiently deep to penetrate the *stratum corneum* but not deep enough to produce bleeding.
Patching technique:	Dermal application onto clipped sites, of which two were abraded and two were intact (4 test sites in total). Test sites were wrapped in an occlusive dressing consisting of 1 inch square gauze patch and a wrap of latex rubber on top held in place by an elastic tape.
Chemical preparation:	Test article was used undiluted.
Chemical application:	Test article (0.5 mL glyphosate) was topically applied to four test sites of each animal and wrapped by an occlusive dressing.
Chemical removal:	Exposure sites wiped to remove excess test material.

B: Study design and methods

In life dates: 1980-08-26 to 1980-08-29

Animal assignment and treatment:

The test was conducted using three male and three female young adult New Zealand White rabbits. The skin of the dorsal surface of each animal was shaved with an electric clipper prior to the administration of the test material. Test material (0.5 mL MON 0139) was applied to four test sites (two abraded and two non-abraded) on the dorsal part of each rabbit under one-inch square gauze patches, covered by means of an occlusive dressing consisting of latex rubber wrap, which was held in place by an elastic band. After 24-hours of exposure, the occlusive dressing was removed, and the skin was wiped to remove any excess of test material.

Skin reactions were assessed 24 and 72-hours after removal of the patches. Skin reactions were scored according to the Draize method.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Clinical observations were not recorded.

C. BODY WEIGHT

Body weight and body weight gain were not recorded.

D. NECROPSY

No necropsies were performed.

E. SKIN OBSERVATIONS

Very slight erythema (grade 1) was observed in 1/6 animals after 24-hours at one of two test sites with intact skin. No oedema was observed at the application sites of any animal at any observation time point. After 72-hours no erythema was observed in any of the animals.

Due to the lack of scoring data at the 48 hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case).

The calculated mean individual scores over the 24 - 72-hours readings were 0.67 for one animal and 0.0 for the remaining 5 animals for erythema and 0.0 for oedema.

Table 6.2.4.23-18 Primary skin irritation of MON 0139 to rabbits [redacted] 1981): Skin irritation scores of intact skin (individual values)

Evaluation interval	Animal No.	Erythema R/L[§]	Oedema R/L
24-hours	01M01 (♂)	0/1	0/0
	01M02 (♂)	0/0	0/0
	01M03 (♂)	0/0	0/0
	01F01 (♀)	0/0	0/0
	01F02 (♀)	0/0	0/0
	01F03 (♀)	0/0	0/0

72-hours	01M01 (♂)	0/0	0/0
	01M02 (♂)	0/0	0/0
	01M03 (♂)	0/0	0/0
	01F01 (♀)	0/0	0/0
	01F02 (♀)	0/0	0/0
	01F03 (♀)	0/0	0/0
Individual 24-72 h means[#]	**01M01 (♂)**	**0.67***	**0.0**
	01M02 (♂)	**0.0**	**0.0**
	01M03 (♂)	**0.0**	**0.0**
	01F01 (♀)	**0.0**	**0.0**
	01F02 (♀)	**0.0**	**0.0**
	01F03 (♀)	**0.0**	**0.0**

§ Two test sites have been evaluated (right test site (R) and left test site (L))
[#] For calculation, persistence of 24-hour scores over 48 hours is assumed
* Calculation based on score 1 at one test site, mean calculated per test site.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, six instead of three animals, 24-hours exposure instead of 4 hours, occlusive instead of semi-occlusive conditions, no scoring of skin effects at reading time points of 48 hours after application.
As the occlusive application and the 24-hour exposure are considered to represent a worst-case scenario, these deviations do not compromise the negative study outcome. Nevertheless, due to the deviations, the study is considered to be used as supplemental information. Therefore, the study is presented here for the sake of completeness, only.
The occlusive application of glyphosate to rabbit skin for 24-hours elicited only slight skin reactions at the application site of one animal at one observation time. The mean individual score over 24 - 72-hours was 0.67 for erythema for one animal (assuming persistence of the 24-hour score over 48 hours) and 0.0 for six animals and 0.0 for oedema for all animals. Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:
The study is considered unacceptable since the study was not performed according to GLP and the study reporting is very limited. Therefore, no conclusion could be drawn.

B.6.2.4.24. Study 24

Data point:	CA 5.2.4/024
Report author	[redacted]
Report year	1979
Report title	Primary Dermal Irritation Study in Rabbits
Report No	[redacted] 77-428
Document No	Not reported
Guidelines followed in study	None; study conducted prior to test guidelines existence

Deviations from current test guideline (OECD 404, 2015)	Six instead of three rabbits were used, no step-wise approach, animals were treated simultaneously. Rationale for *in vivo* testing and consideration of pre-existing data was not documented. No time point is mentioned when the skin was clipped. In addition, abraded skin was exposed. The test item was administered as 0.125 per test site, with four test sites per animal (total of 0.5 g) rather than one dose of 0.5 g to one site. The test item was administered for 24-hours instead of 4 hours and occlusive (Dermicel® tape wrapped with plastic sheeting) instead of semi-occlusive dressing. No data on removal of residual test material was reported. Signs of dermal irritation were recorded only at 24 and 72-hours. No clinical signs and body weights were reported. Additionally, no information was provided about the stability of the test item, food and drinking, the humidity and housing, age, acclimation period of the rabbits.
Previous evaluation	Yes, not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	**Conclusion GRG:** Supportive (no GLP-study, no guideline, clinical signs & body weight not reported), Category 2a **Conclusion AGG:** The study is considered unacceptable due to limited reporting

Glyphosate (Batch: XLI-180, Purity: 99 %) was evaluated for potential primary dermal irritation using six New Zealand White rabbits (three males and three females). The skin was clipped over the back and sides. Each rabbit was administered 0.5 mL of the test article (applied as 25 % w/v solution in distilled water, equivalent to 0.125 g glyphosate per test site) to two intact test sites and two abraded sites. Each test site was covered occlusive for 24-hours following dose administration. Dermal irritation was scored according to the Draize method at 24 and 72-hours after patch removal.

In the intact skin, one male had very slight erythema (one site) at 24-hours following test substance application. The irritation was recovered at the 72-hour time point. There was no other irritation noted at 24 or 72-hours for the other animals.

Due to the lack of scoring data at the 48-hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case). The calculated mean individual scores (per site) were 0.67 for one male and 0.0 for the remaining animals for erythema, and 0.0 for oedema all animals. Thus, glyphosate is considered not irritant.

Due to the deviations from the current OECD TG 404 (2015), the study can be used as supportive information only.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XLI-180
Purity:	99 %
Stability of test compound:	Not specified

2. **Vehicle and/or positive control:** Distilled water
3. **Test animals:**

Species:	Rabbit	
Strain:	New Zealand White	
Source:	[redacted]	
Age:	Not specified	
Sex:	Male and female	
Weight at dosing:	2.25 – 2.80 kg	
Acclimation period:	Not specified	
Diet/Food:	Not specified	
Water:	Not specified	
Housing:	Not specified	
Environmental conditions:	Temperature:	Not specified
	Humidity:	Not specified
	Air changes:	Not specified
	Photocycle:	Not specified

4. Test conditions:

Patch site preparation technique:	The back and sides were closely clipped with an electric clipper. Four test sites per rabbit, each site 1" × 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact.
Patching technique:	Test substance was applied beneath a surgical gauze square, 1" × 1", eight single layers thick, placed directly on the test site and secured with Dermicel® tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material (occlusive dressing).
Chemical preparation:	25 % w/v solution in distilled water
Chemical application:	0.5 mL / test site [equivalent to 0.125 g glyphosate / test site]
Chemical removal:	After 24-hours the sheeting and gauze patches were removed. No removal of test substance reported.

B: Study design and methods

In life dates: Not specified

Animal assignment and treatment:
Six albino rabbits were closely clipped over the back and sides with an electric clipper. There were four test sites per rabbit, each site 1" × 1" in area. Two sites, one on each side of the spinal column were abraded, while the remaining two sites were left intact. The abrasions were sufficiently deep so as to penetrate the stratum corneum, but not so deep as to disturb the derma or produce bleeding.

The test material was administered as a 25 % w/v solution in distilled water. In all cases 0.5 mL of the test substance was applied beneath a surgical gauze square, 1" × 1", eight single layers thick, placed directly on the test site and secured with Dermicel® tape. The animals were then wrapped with plastic sheeting secured with masking tape to help contain the test material. After 24-hours' exposure period the occlusive dressing was removed.

Observations for signs of dermal irritation or systemic toxicity were recorded at 24 and 72-hours after application according to the table below. At each observation all treated sites were scored for erythema, oedema, and eschar formation.

Skin reaction grading according to Draize criteria used by [redacted] (1979)

Skin Reaction	Grading
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef 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 (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

Not reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

No necropsy was performed.

E. SKIN OBSERVATIONS

One male had very slight erythema (one site) at 24-hours following test substance application. The irritation was recovered at the 72-hour time point. There was no other irritation noted at 24 or 72-hours for the other animals.

Due to the lack of scoring data at the 48 hour time point, for the calculation of the mean individual score over 24 - 72-hours (as required for classification purpose according to Regulation (EC) 1272/2008), persistence of irritating effects over the 48 hour time point was assumed (worst case). The calculated mean individual scores (per site) were 0.67 for one male and 0.0 for the remaining animals for erythema, and 0.0 for oedema all animals.

Table 6.2.4.24-19 Primary Dermal Irritation Study in Rabbits [redacted] 1979): Skin irritation scores of intact skin (individual values)

Evaluation interval	Animal No.	Erythema A/B[§]	Oedema A/B[§]
24-hours	655 (♀)	0/0	0/0
	657 (♂)	0/1	0/0
	658 (♀)	0/0	0/0
	659 (♂)	0/0	0/0
	660 (♂)	0/0	0/0
	661 (♀)	0/0	0/0
72-hours	655 (♀)	0/0	0/0

	657 (♂)	0/0	0/0
	658 (♀)	0/0	0/0
	659 (♂)	0/0	0/0
	660 (♂)	0/0	0/0
	661 (♀)	0/0	0/0
Individual 24 – 72 h means[#]	**655 (♀)**	**0.0**	**0.0**
	657 (♂)	**0.67[*]**	**0.0**
	658 (♀)	**0.0**	**0.0**
	659 (♂)	**0.0**	**0.0**
	660 (♂)	**0.0**	**0.0**
	661 (♀)	**0.0**	**0.0**

§ Two test sites have been evaluated (test site A and test site B)

[#] For calculation, persistence of 24-hour scores over 48 hours is assumed

* Calculation based on score 1 at one test site, mean calculated per test site.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Compared to the current OECD TG 404 (2015), deviations such as no step-wise approach, use of 6 instead of 3 animal, occlusive instead of semi-occlusive dressing, exposure 24 instead of 4 hours, record of skin reactions at 24 and 72-hours, only, no report of body weights and clinical signs, no information about food and water supply, housing and environmental conditions of the rabbits are noted.

Due to the deviations from the current OECD TG 404 (2015), the non-GLP study not following any guideline can be used as supplementary information, only.

Occlusive application of glyphosate to intact rabbit skin for 24-hours elicited skin reactions at the application site of two animals at the 24-hour observation time. The calculated individual mean score for 24 - 72-hours was 0.67 (assuming persistence of the 24-hour score over 48 hours) for one male for erythema and 0.0 for the remaining animals for erythema and all oedema values, respectively.

Thus, glyphosate is not considered a skin irritant.

Assessment and conclusion by RMS:

The study is considered unacceptable since the study was not performed according to GLP and the study reporting is very limited. Therefore, no conclusion could be drawn. This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.2.5. Eye irritation

B.6.2.5.1. Study 1

Data point:	CA 5.2.5/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Acute eye irritation study in rabbits
Report No	10/218-005N
Document No	NOT REPORTED
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), 2004/73/EC B.5 (2008)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Based on the information provided by the Sponsor, treatment was performed even though the pH of glyphosate technical was measured at 1.99. The relative humidity was out of the guideline range during the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered acceptable although it is noted that due to the low pH the study should not have been conducted.

In a primary eye irritation study, 0.1 g of glyphosate technical (Batch: 569753, Purity: 96.3 %) was instilled into the conjunctival sac of the left eye of one adult male New Zealand White rabbit. The untreated right eye served as control. Immediately following instillation, an assessment of initial pain was made. Scoring of irritation effects was performed approximately 1 and 24-hours after test material instillation. Irritation was scored according to the Draize numerical evaluation.

Initial Pain Reaction (IPR) scores were taken after instillation into the eye and a score of 3 (on a 0 – 5 scale) was observed. Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit 1 and 24-hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva and dry surface of the eye were noted at one hour after the treatment. Fluorescein staining was positive at the 24-hour observation. No clinical signs of systemic toxicity were observed in the animal during the study and no mortality occurred.

Based on the symptoms, no further animals were dosed and the study was terminated after the 24-hour observation (Regulation (EC) No 440/2008).

Glyphosate technical is considered to cause serious damage/corrosion to the eyes under the chosen test conditions.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate technical
Description:	Dry white powder
Lot/Batch number:	569753
Purity:	96.3 % w/w
Stability of test compound:	Stable under storage conditions are <30 °C, Recertification date end of August 2011

2. **Vehicle and/or positive control:** None
3. **Test animals:**

Species:	Rabbit	
Strain:	New Zealand White	
Source:	[redacted]	
Age at dosing:	Approximately 14 weeks	
Sex:	Male	
Weight at dosing:	3035 g	
Housing:	Individually in metal cage	
Acclimation period:	13 days	
Diet:	Purina Base – Lap gr. diet (Agribrands Europe Hungary PLC, H-5300 Karcag, Madarasi út, Hungary), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	24 – 64 %
	Air changes:	15 – 20 / hour
	Photoperiod:	12 hours light / dark cycle

B: Study design and methods

In-life dates: 2011-12-21 to 2011-12-22

Animal assignment and treatment:

Approximately 1 hour before the start of the test, the eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect using a hand-held slit-lamp. The animal used in the study was free of ocular damage. Initially, a single rabbit was treated.

An amount of 0.1 g of the test material was placed into the conjunctival sac of the left eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about 1 second immediately after treatment, to prevent loss of the test material, and then released. The right eye remained untreated and was therefore used as control.

Immediately after administration of the test material, an assessment of the initial pain reaction was made according to a 0-5 scale. Following review of the ocular responses produced in the first treated animal, no further animals were treated. The treated eyes were not rinsed after instillation.

The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed approximately 1 and 24-hours following treatment, according to the numerical evaluation described by Draize. The treated eye was further examined using 2 % fluorescein solution before treatment and 24-hours after treatment. Additionally, any other signs of eye irritation were recorded.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animal during the study.

C. BODY WEIGHT

The body weight was considered to be within the normal range of variability.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

An initial pain reaction score of 3 (on a 0 – 5 scale) was recorded.

Conjunctival redness, chemosis, and conjunctival discharge, as well as corneal opacity, were observed in the rabbit 1 and 24-hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, a few black points on the conjunctiva, and dry surface of the eye were noted one hour after the treatment. Fluorescein staining was positive at the 24-hours' observation.

Based on the symptoms, no further animals were tested and the study was terminated after the 24-hour observation (Regulation (EC) No 440/2008).

Table 6.2.5.1-1 Glyphosate technical: Acute eye irritation study in rabbits. [redacted] 2011): Eye irritation – Individual irritation scores

Time	Cornea		Iris	Conjunctiva		
	Opacity	Area		Redness	Chemosis	Discharge
1 hour	2	4	0	2	3	3
24-hours	3	3	1	3	4	3
Individual mean (1, 24 h)	**2.5**	**--**	**0.5**	**2.5**	**3.5**	**--**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and the use of test material with a pH of 1.99, the study is in concordance with the current OECD TG 405 (2017). The deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate technical into the rabbit eye elicited strong response in treated animal. The individual scores at 24-hours for the animal were 3 for corneal opacity, 1 for iris lesions, 3 for conjunctival redness, and 4 for conjunctival chemosis, and therefore, the study was terminated at 24-hours.
Thus, under test conditions of the study, glyphosate technical is considered to cause serious damage/corrosion to the eyes.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable. The pH of the test substance is 1.99 and eye damage is seen in the treated rabbit. Therefore, the test substance is considered as corrosive to the rabbit eye under the test conditions.

B.6.2.5.2. Study 2

Data point:	CA 5.2.5/002
Report author	[redacted]
Report year	2010
Report title	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Report No	24606
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), EC method B.5. (2004/73/EC)

Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Himalayan rabbits are used instead of the New Zealand White. The number of air changes was not specified. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

In an eye irritation study, 0.1 g of the undiluted, solid glyphosate TC (Batch: 20090506, Purity: 97.3 %) was instilled into the right conjunctival sac of three male Himalayan rabbits in a stepwise manner. One hour after instillation the eyes were rinsed with sodium chloride solution. Animals were observed for 7 days. Eye irritation was scored 1, 24, 48 and 72-hours and 4 and 7 days after test item instillation. Body weight of all animals was measured at the beginning and at the end of the study. Behaviour and food consumption were monitored.

Corneal opacity (grade 1) was observed in all animals 24 – 72-hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24-hours after instillation revealed cornea1 staining in all animals (½ to ¾ of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72-hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 1 hour – 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 1 hour and 24-hours after instillation. In addition, secretion was observed in all animals 1 hour and 24-hours after instillation. These effects were fully reversible within 7 days. There were no systemic intolerance reactions.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00, 1.00
- for iris lesions: 0.67, 0.67, 1.00
- for conjunctival redness: 1.00, 1.33, 2.00
- for conjunctival chemosis: 0.33, 0.33, 0.33

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3 % (w/w)
Stability of test compound:	Expiry date: 2011-05 (when stored at room temperature in the dark)

2. **Vehicle and/ or positive control:** No vehicle was used
3. **Test animals:**

Species:	Rabbit
Strain:	Himalayan

Source:	[redacted]
Age:	Approximately 6 – 8 months
Sex:	Males
Weight at dosing:	2.5 – 2.7 kg
Acclimation period:	At least 20 adaptation days
Diet/Food:	ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period
Water:	Tap water, *ad libitum*, before and after the exposure period
Housing:	The animals were kept singly in cages measuring 380 mm × 425 mm × 600 mm (manufacturer: Dipl. Ing. W. EHRET GmbH, 16352 Schönwalde, Germany). After test item application, animals were held in restrainers for 8 hours to prevent a complete body turn, wiping of the eyes with paws, and excluded irritation by excrements/urine.
Environmental conditions:	Temperature: 20 ± 3 °C
	Humidity: 30 – 70 %
	Air changes: Not reported
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-10-26 to 2009-11-12 (Start of treatment to study completion)

Animal assignment and treatment:
A quantity of 0.1 g of the test item was administered into one eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, two further animals were employed 24-hours after start of the initial test.

One hour after instillation the eyes were rinsed with 20 mL sodium chloride solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and 1, 24, 48, 72-hours and 4 – 7 days after the administration. Twenty-four hours and 7 days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions. The eye reactions were observed, registered and scored according to the Draize scheme (see table below).

Numerical scoring system (grading of ocular lesions)

Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, iris reacting to light (a sluggish reaction is considered to be an effect)	1
Haemorrhage, gross destruction, or no reaction to light	2
Conjunctivae, Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2

Diffuse beefy red	3
Conjunctivae, Chemosis (refers to lids and/or nictitating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4

* The area of corneal opacity should be noted

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No data on necropsy were reported.

E. EYE OBSERVATIONS

Corneal opacity (grade 1) was observed in all animals 24 – 72-hours, in animal no. 1 until 4 days and in animal no. 3 until 5 days after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in all animals (½ – ¾ of the surface). Irritation of the iris (grade 1) was observed in all animals 24 and 48 hours, in animal no. 3 until 72-hours after instillation. Conjunctival redness (grade 1 or 2) was observed in all animals 1 hour – 4 days, in animal no. 3 until 6 days after instillation. Chemosis (grade 1) was observed in all animals 1 hour and 24-hours after instillation. In addition, secretion was observed in all animals 1 hour and 24-hours after instillation.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were 1.00 for all animals for corneal opacity, 0.67, 0.67, 1.00 for iris lesions, 1.00, 1.33, 2.00 for conjunctival redness and 0.33 for all animals for conjunctival chemosis. These effects were fully reversible within 7 days.

Table 6.2.5.2-1 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. [redacted] 2010): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1 (male)	1	0	0	1[a]	1
	24	**1**	**1**	**1[a]**	**1**
	48	**1**	**1**	**1**	**0**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	0	0	0	0
	Day 6	--	--	--	--
	Day 7	--	--	--	--
Individual mean (24, 48, 72 h)		**1.00**	**0.67**	**1.00**	**0.33**

Table 6.2.5.2-1 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted], 2010): Eye irritation – Individual irritation scores

Rabbit 2 (male)	1	0	0	1[a]	1
	24	**1**	**1**	**2[a]**	**1**
	48	**1**	**1**	**1**	**0**
	72	**1**	**0**	**1**	**0**
	Day 4	0	0	1	0
	Day 5	0	0	0	0
	Day 6	--	--	--	--
	Day 7	--	--	--	--
Individual mean (24, 48, 72 h)		**1.00**	**0.67**	**1.33**	**0.33**
Rabbit 3 (male)	1	0	0	1[a]	1
	24	**1**	**1**	**2[a]**	**1**
	48	**1**	**1**	**2**	**0**
	72	**1**	**1**	**2**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	1	0
	Day 6	0	0	1	0
	Day 7	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**1.00**	**2.00**	**0.33**

[a] = secretion; -- = no examination

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals/animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate TC into the rabbit eye elicited slight to moderate irritation in all animals. The mean scores were 1.00 for cornea opacity for all animals, 0.67, 0.67, 1.0 for iris effects, 1.00, 1.33, 2.00 for conjunctivae redness, and 0.33 for chemosis for all animals. These effects were fully reversible within 7 days. Thus, glyphosate is considered irritant to the eye.

Nevertheless, for classification purposes all eye irritation studies should be taken into account.

Assessment and conclusion by RMS:

It is agreed with the applicant's conclusion. Based on the findings of this study, glyphosate is considered irritant to the eye.

The study was considered supplementary in the previous evaluation (RAR, 2015) due to the washing of the eyes after one hour. However, it should be noted that the test substance is a solid and therefore washing with a saline solution after 1 hour is permitted.

B.6.2.5.3. Study 3

Data point:	CA 5.2.5/003
Report author	[redacted]
Report year	2009
Report title	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Report No	24878
Document No	Not reported
Guidelines followed in study	OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), OPPTS 870.2400 (1998)
Deviations from current test	No treatment with systemic analgesic or topical anaesthesia of the animals /

guideline (OECD 405, 2017)	animal eyes prior, during or after test substance application. Himalayan rabbits are used instead of the New Zealand White. The number of air changes was not specified. This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

In an eye irritation study, 0.1 g of the undiluted test substance glyphosate TC (Batch: 2009051501, Purity: 96.4 %) was instilled into the right conjunctival sac of three young male adult Himalayan rabbits. Animals were observed for 8 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72-hours and 4, 5, 6, 7 and 8 days after test item instillation. Application of glyphosate TC into the rabbit eye resulted in slight and fully reversible ocular changes, not exceeding Grade 1. All eye effects were reversible within 8 days after instillation. No signs of corrosion or staining were observed in any eye. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00, 1.00
- for iris lesions: 1.00, 0.67, 0.33
- for conjunctival redness: 1.00, 1.00, 1.00
- for chemosis of the conjunctiva: 0.67, 0.33, 0.00

Based on the study, glyphosate TC has the potential for irritating effects to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	Expiry date: 15 May 2011

2. **Vehicle and/ or positive control:** None

3. **Test animals:**

Species:	Rabbit
Strain:	Himalayan
Source:	[redacted]
Age:	Approximately 6.5 – 7.5 months
Sex:	Males
Weight at dosing:	2.5 – 2.8 kg
Acclimation period:	At least 20 days

Diet/Food:	ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period	
Water:	Tap water, *ad libitum*	
Housing:	Individual housing during acclimatisation period and for study period starting 8 hours after test item instillation. At test item instillation animals were kept in restrainers for 8 hours to prevent wiping of the eyes and exposure to urine or excrements.	
Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 – 70 %
	Air changes:	Not reported
	Photoperiod:	12 hours light / dark cycle

B: Study design and methods

In life dates: 2009-10-15 to 2009-10-29

Animal assignment and treatment:

The test was conducted using three young male adult Himalayan albino rabbits. The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the right eye of the rabbits. The lids were then gently held together for about one second. One hour after instillation the eyes were rinsed with 20 mL saline solution. The left eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72-hours, as well as 4 – 8 days after instillation. Twenty-four hours and seven days after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions. Additionally, behaviour and food consumption were monitored. Body weights were determined at beginning of the study and at termination.

Numerical scoring system (Draize scale for scoring ocular irritation)

Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, or injection, iris reactive to light (a sluggish reaction is considered to be an effect)	1
Haemorrhage, gross destruction, or no reaction to light	2
Conjunctivae, Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1
Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse, beefy red	3
Conjunctivae, Chemosis (lids and/or nictitating membranes)	
Normal	0
Some swelling above normal	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

No adverse effects on body weight were noted.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Corneal opacity (grade 1) was observed in all animals 24 – 72-hours, in animal no. 2 until 4 days and in animal no. 1 until 7 days after instillation. The fluorescein test demonstrated corneal staining in all animals when performed 24-hours after instillation and only in animal no. 1 when performed 7 days after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in animal nos. 1 and 2 (½ – ¾ of the surface) and in animal no. 3 (¼ – ½ of the surface). The fluorescein test performed 7 days after instillation revealed staining in animal no. 1 (up to ¼ of the surface).
Irritation of the iris (grade 1) was observed in all animals at the 24-hour observation, in animal no. 2 until 48 hours and in animal no. 1 until 72-hours after test item instillation.

Conjunctival redness (grade 1) was noticed in all animals in the observation period from 1 hour to 72-hours as well as in animal no. 1 until 4 days and in animal no. 2 until 5 days after instillation.
Chemosis (grade 1) was observed in all animals at 1 hour, in animal no. 2 until 24-hours, and in animal no. 1 until 48 hours after instillation.

In addition, secretion was observed in all animals 1 hour after instillation. There were no systemic reactions. All rabbits were free of ocular signs by Day 8 after instillation.

The individual mean irritation scores (24 – 72-hours) were calculated to be 1.00 for corneal opacity and for conjunctiva redness for all three animals, 1.00, 0.67 and 0.33 for iris lesions, and 0.67, 0.33 and 0.00 for chemosis of the conjunctiva (see table below).

Table 6.2.5.3-1 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. [redacted] 2009): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1 (male)	1	0	0	1	1[a]
	24	**1**	**1**	**1**	**1**
	48	**1**	**1**	**1**	**1**
	72	**1**	**1**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	0	0
	Day 6	1	0	0	0
	Day 7	1	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**1.00**	**1.00**	**0.67**
Rabbit 2 (male)	1	0	0	1	1[a]
	24	**1**	**1**	**1**	**1**
	48	**1**	**1**	**1**	**0**

Table 6.2.5.3-1 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. ([redacted]. 2009): Eye irritation in rabbits – Individual irritation scores

	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	0	0	1	0
	Day 6	0	0	0	0
	Day 7	—	—	—	—
	Day 8	—	—	—	—
Individual mean (24, 48, 72 h)		**1.00**	**0.67**	**1.00**	**0.33**
Rabbit 3 (male)	1	0	0	1	1[a]
	24	**1**	**1**	**1**	**0**
	48	**1**	**0**	**1**	**0**
	72	**1**	**0**	**1**	**0**
	Day 4	0	0	0	0
	Day 5	—	—	—	—
	Day 6	—	—	—	—
	Day 7	—	—	—	—
	Day 8	—	—	—	—
Individual mean (24, 48, 72 h)		**1.00**	**0.33**	**1.00**	**0.00**

[a] secretion

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid.

A single instillation of glyphosate into the rabbit eye revealed slight and fully reversible ocular changes (≤ grade 1); all eye effects were reversible within 8 days after instillation.

It is concluded that the test item is 'irritant' to the eyes of Himalayan rabbits based on the individual mean scores over 24, 48 and 72 h, which were 1.00 for all animals for the endpoints corneal opacity and conjunctival redness, 0.33, 0.67, 1.00 for iris lesions and 0.00, 0.33, 0.67 for chemosis. Nevertheless, for classification purposes the serious eye damage observed in another study should be taken into account.

Assessment and conclusion by RMS:

It is agreed with the applicant's conclusion. Based on the findings of this study, glyphosate is considered irritant to the eye.

The study was considered supplementary in the previous evaluation (RAR, 2015) due to the washing of the eyes after one hour. However, it should be noted that the test substance is a solid and therefore washing with a saline solution after 1 hour is permitted.

B.6.2.5.4. Study 4

Data point:	CA 5.2.5/004
Report author	[redacted]
Report year	2009
Report title	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits
Report No	23914
Document No	NOT REPORTED
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), EC method B.5 (2004/73/EC)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. The number of air changes was not specified. This deviation is not considered to affect the

	study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

Under the present test conditions, a single instillation of 0.1 g glyphosate TC (Batch: 20080801, Purity: 98.8 %) per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:

Corneal opacity (grade 1) was observed in animal no. 1 (24 – 72-hours) and in animal no. 3 (24 and 48 hours) after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in animal no. 1 and 3 (up to 1/4 of the surface).

Conjunctival redness (grade 1) was observed in all animals 60 minutes – 48 hours, in animal no. 1 until 72-hours after instillation.

Chemosis (grade 1) was observed in animal no. 1 (24 and 48 hours) after instillation. In addition, secretion was observed in all animals 60 minutes after instillation. The irises were not affected by instillation of the test item.

There were no systemic reactions. All animals were free of signs of ocular irritation by Day 4.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 0,00, 0.67
- for iris lesions: 0.00, 0.00, 0.00
- for conjunctival redness: 1.00, 0.67, 0.67
- for conjunctival chemosis: 0.67, 0.00, 0.00

Based on the study, glyphosate TC was not irritating to the eyes under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	Expiry date: 2010-08-01

2. **Vehicle and/or positive control:** No vehicle used
3. **Test animals:**

Species:	Rabbit
Strain:	Himalayan

Source:	[redacted]	
Age:	Approximately 4 – 32.5 months at dosing	
Sex:	Males	
Weight at dosing:	3.9 – 4.1 kg	
Acclimation period:	At least 20 days	
Diet/Food:	ssniff® K-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum* before and after the exposure period	
Water:	Tap water, *ad libitum*	
Housing:	Individual housing during acclimatisation period and for study period starting 8 hours after test item instillation. After test item instillation, animals were kept in restrainers for 8 hours to prevent wiping of the eyes and exposure to urine or excrements.	
Environmental conditions:	Temperature:	20 ± 3 °C
	Humidity:	30 – 70 %
	Air changes:	Not reported
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2009-02-04 to 2009-02-15 (Start of treatment to study completion)

Animal assignment and treatment:
A quantity of 0.1 g of the test item was administered into the right eye each of three animals. The test item was placed into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of the material. The left eye, which remained untreated, served as a control. The test was performed initially using one animal. As no corrosive or severe irritant effects were observed in this animal, two further animals were employed 24-hours after start of the initial test.

One hour after instillation the eyes were rinsed with 20 mL sodium chloride solution. The eyes were examined ophthalmoscopically with a slit lamp prior to the administration and l, 24, 48, 72-hours and 4 days after the administration. The eye reactions were observed and registered. 24-hours after administration, fluorescein was applied to the eyes before being examined to aid evaluation of the cornea for possible lesions.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

A single instillation of 0.1 g test item per animal into the conjunctival sac of the right eye of three rabbits caused the following changes:

Corneal opacity (grade 1) was observed in animal no. 1 (24 – 72) hours and in animal no. 3 (24 and 48 hours) after instillation. The fluorescein test performed 24-hours after instillation revealed corneal staining in animal no. 1 and 3 (up to 1/4 of the surface).

Conjunctival redness (grade 1) was observed in all animals 60 – 48 hours, in animal no. 1 until 72-hours after instillation.

Chemosis (grade 1) was observed in animal no. 1 (24 and 48 hours) after instillation.

In addition, secretion was observed in all animals 60 minutes after instillation.

The irises were not affected by instillation of the test item and there were no systemic reactions.

All animals were free of signs of ocular irritation by Day 4.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were 1.00, 0,00, 0.67 for corneal opacity, 0.00 for all animals for iris lesions, 1.00, 0.67, 0.67 for conjunctival redness and 0.67, 0.00, 0.00 for conjunctival chemosis.

Table 6.2.5.4-1 Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits. [redacted] 2009): Eye irritation with eye irrigation 1 hour after application – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
Rabbit 1	1	0	0	1[a]	0
	24	**1**	**0**	**1**	**1**
	48	**1**	**0**	**1**	**1**
	72	**1**	**0**	**1**	**0**
	Day 4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**0.00**	**1.00**	**0.67**
Rabbit 2	1	0	0	1[a]	0
	24	**0**	**0**	**1**	**0**
	48	**0**	**0**	**1**	**0**
	72	**0**	**0**	**0**	**0**
	Day 4	--	--	--	--
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.67**	**0.00**
Rabbit 3	1	0	0	1[a]	0
	24	**1**	**0**	**1**	**0**
	48	**1**	**0**	**1**	**0**
	72	**0**	**0**	**0**	**0**
	Day 4	--	--	--	--
Individual mean (24, 48, 72 h)		**0.67**	**0.00**	**0.67**	**0.00**

[a] = secretion; -- = no examination

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study follows the current OECD TG 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate TC into the rabbit eye elicited slight response in treated animals. The individual mean scores over 24, 48 and 72-hours for the three animals were 1.00, 0,00, 0.67 for corneal opacity, 0.0 for all animals for iris lesions, 1.00, 0.67, 0.67 for conjunctival redness, and 0.67, 0.00, 0.00 for conjunctival chemosis.

Thus, under test conditions of the study, glyphosate technical is considered not irritating to the eye mucosa of

rabbits.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Based on the findings of this study, glyphosate is considered not irritant to the eye.
The study was considered supplementary in the previous evaluation (RAR, 2015) due to the washing of the eyes after one hour. However, it should be noted that the test substance is a solid and therefore washing with a saline solution after 1 hour is permitted.

B.6.2.5.5. Study 5

Data point:	CA 5.2.5/005
Report author	[redacted]
Report year	2009
Report title	Expert Statement Glyphosate technical: Primary eye irritation study in rabbits
Report No	C22897
Document No	Not reported
Guidelines followed in study	OECD 405 (2002) Council Regulation (EC) No 440/2008 (2008)
Deviations from current test guideline (OECD 405, 2017)	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered acceptable

A pH measurement was performed with the test item in a 1% (w/w) (Glyphosate technical; batch GI-1045; purity 96.66% reported in CA 5.2.2/005) solution in purified water before the study initiation. The pH of the test item was found to be 1.93.

According to the OECD Guidelines 405 and Council Regulation (EC) No 440/2008 B.5:
Physicochemical properties and chemical reactivity – Substances exhibiting pH extremes such as ≤2.0 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid reserve (buffering capacity) may also be taken into consideration.

It is assumed that the test substance item has corrosive properties; therefore, no eye irritation study in rabbits with glyphosate technical was performed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Based on the extreme pH, the test item requires classification as Eye damaging, Category 1, H318 (Causes serious eye damage) according to the criteria laid down in the CLP Regulation (EC No. 1272/2008).

Assessment and conclusion by RMS:
Agreed with the applicant and the statement provided by the Notifiers. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.5.6. Study 6

Data point:	CA 5.2.5/006
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Acute Eye Irritation Study in Rabbits
Report No	12172-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.2400, Equivalent to OECD 405 (2002)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Temperature and humidity were outside the recommended range (16 – 23 °C instead of 20 ± 3 °C and 33 – 92 % instead of 30 – 70 %). The stability of the test chemical is not reported. All animals were treated at once instead of stepwise, as requested by the current OECD guideline 405 (2017). Systemic adverse effects and record of clinical signs were not performed. These deviations did not affect the study outcome.
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

An acute eye irritation study was conducted on three albino rabbits using test substance glyphosate (Batch: 080704-1 thru 5, Purity: 96.4 %). The test substance, 0.1 mL by volume (93.2 mg), was placed into the conjunctival sac of the right eye of each animal. All treated eyes were washed with water after recording the 24-hour observation. Eye irritation was scored 1, 24, 48 and 72-hours and 4, 7, 10, 14 and 17 days after test item instillation. No further observations on clinical signs were recorded.

Grades for opacity were ranging from 0 (after 1 hour) to grade 1 or 2 from 24-hour to Day 4, returning to score 0 at Day 7 and 10 for two animals, respectively. One animal did not show any effect on opacity. The iris grading was 1 between Day 1 to 4 in only one animal and did not show any effect in the remaining two rabbits. Conjunctival redness was noted one hour after removal of test item (score 1) in all three animals. An increase to grade 2 was observed in two animals at 24-hours, which decreased to grade 1 at Day 4 and Day 7, respectively. Clearance of redness (score 0) was observed after 72-hours (one animal) and at Day 17 (two animals). For chemosis, the highest grade observed was 3 for one animal between Days 1 and 3. In the other two animals the effect was not as pronounced and fully reversible in all animals latest at Day 17. Discharge was also observed with the highest grade 3 on Day 1 but was also fully reversible in all animals latest at Day 7. No necrosis or ulceration was seen in any of the treated animals. All effects observed in any eye were fully reversible within 7 days.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 0.00, 2.00
- for iris lesions: 0.00, 0.00, 1.00
- for conjunctival redness: 2.00, 0.67, 2.00
- for chemosis of the conjunctiva: 1.67, 0.00, 3.00

Based on the study, glyphosate TC has irritating properties onto the eye under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4 % (2008-10-17); 96.71 % (2009-01-08)
Stability of test compound:	No data given in the report

2. **Vehicle and/ or positive control:** No vehicle was used

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 3 months
Sex:	Males (2) and female (1; nulliparous and non-pregnant)
Weight at dosing:	Males: 2.2 – 2.4 kg; female: 2.3 kg
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Lab Rabbit Diet #5321, 8 oz. daily
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 16 – 23 °C *
	Humidity: 33 – 92 % *
	Air changes: 10 – 12 / hour
	Photoperiod: 12-hour light / dark cycle
	* Temperature and humidity were outside of protocol range, but did not affect study outcome.

B: Study design and methods

In life dates: 2008-11-08 to 2008-11-27 (Start of treatment to study completion)

Animal assignment and treatment:
Healthy albino rabbits were released from quarantine and both eyes of each animal were carefully examined within 24-hours prior to treatment with a fluorescein sodium ophthalmic solution and cobalt-filtered light. Both eyes of each animal were again carefully examined just prior to treatment, but without the fluorescein sodium ophthalmic solution. Only those animals without eye defects or irritation were selected for testing. On Day 0, a dose of 0.1 mL by volume (93.2 mg) of the undiluted test substance was placed into the conjunctival sac of the right eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance was dropped. The lids were gently held together for one second to prevent loss of material. The untreated left eyes served as comparative controls. The grades of ocular reaction were recorded at 1, 24, 48 and 72-hours, and at 4, 7, 10, 14 and 17 days after treatment. The corneas of all treated eyes were examined immediately after the 24-hour observation with a fluorescein sodium ophthalmic solution. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical signs were not recorded.

C. BODY WEIGHT

All animals gained weight over the duration of the study.

D. NECROPSY

No gross abnormalities were recorded at necropsy.

E. EYE OBSERVATIONS

Corneal opacity scores ranged from 0 (after 1 hour) to grade 1 (one animal) or grade 2 (one animal) from 24-hour to Day 4 reading time points, returning to score 0 at Day 7 and 10 for these two animals, respectively. One animal did not show any effect on opacity.

The iris grading was 1 between Day 1 – 4 in only one animal and did not show any effect in the remaining two rabbits.

Conjunctival redness was noted one hour after removal of test item (score 1) in all three animals. An increase to grade 2 was observed in two animals at 24-hours, which decreased to grade 1 at Day 4 and Day 7, respectively. Clearance of redness (score 0) was observed after 72-hours (one animal) and at Day 17 (two animals).

For chemosis, the highest grade observed was 3 for one animal between Days 1 and 3. In the other two animals the effect was not as pronounced and fully reversible in all animals latest at Day 17. Discharge was also observed, with the highest grade 3 on Day 1 but was also fully reversible in all animals latest at Day 7.

Fluorescein staining was observed in two of three eyes at 24-hours after treatment and was not observed in any eyes on Day 10 after treatment. No necrosis or ulceration was seen in any of the treated animals.

All effects observed in any eye were fully reversible within 17 days.

The individual mean irritation scores (24, 48 and 72-hours) of the three rabbits were 1.00, 0.00 and 2.00 for corneal opacity, 0.00, 0.00, 1.00 for iris lesions, 2.00, 0.67, 2.00 for conjunctival redness and 1.67, 0.00, 3.00 for chemosis.

Table 6.2.5.6-1 Glyphosate – Acute Eye Irritation Study in Rabbits. [redacted] 2009): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 3144 (male)	1	0	0	0	1	1	2
	24	**1**	4	**0**	**2**	**2**	2
	48	**1**	4	**0**	**2**	**2**	2
	72	**1**	4	**0**	**2**	**1**	1
	Day 4	1	4	0	1	1	1
	Day 7	1	4	0	1	1	0
	Day 10	0	0	0	1	1	0
	Day 14	0	0	0	1	1	0
	Day 17	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**0.00**	**2.00**	**1.67**	**---**
Rabbit 3202	1	0	0	0	1	1	1
	24	**0**	0	**0**	**1**	**0**	0

Table 6.2.5.6-1 Glyphosate – Acute Eye Irritation Study in Rabbits. ([redacted], 2009): Eye irritation – Individual irritation scores

(male)	**48**	**0**	0	**0**	**1**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
	Day 4	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
	Day 17	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**0.67**	**0.00**	**---**
Rabbit 3201 (female)	1	0	0	0	1	2	2
	24	**2**	4	**1**	**2**	**3**	3
	48	**2**	4	**1**	**2**	**3**	2
	72	**2**	2	**1**	**2**	**3**	1
	Day 4	2	2	1	2	2	1
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	1	0	0
	Day 14	0	0	0	1	0	0
	Day 17	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**3.00**	**---**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate TC into the rabbit eye elicited positive response in all animals. The mean scores were 1.00, 0.00 and 2.00 for corneal opacity, 0.00, 0.00 and 1.00 for iris lesions, 2.00, 0.67 and 2.00 for conjunctival redness and 1.67, 0.00 and 3.00 for chemosis. These effects were fully reversible within 17 days. Thus, glyphosate is considered irritant to the eye.
Nevertheless, for classification purposes all eye irritation studies should be taken into account.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions the test substance is irritating to the rabbit eye. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.5.7. Study 7

Data point:	CA 5.2.5/007
Report author	[redacted]
Report year	2008
Report title	Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical
Report No	[redacted]3996.312.599.07
Document No	Not reported
Guidelines followed in study	OECD 405 (2002)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. The stability of the test chemical is not reported. This deviation is not considered to affect the study outcome.

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

The acute eye irritation / corrosion potential of glyphosate technical (Batch: 20070606, Purity: 98.05 %) was evaluated in New Zealand White rabbits. The test was initially planned for three animals but conducted using one rabbit first. Due to severe ocular reactions observed in the initial test, only one additional animal was tested to confirm the response. One tenth (0.1) g of the undiluted solid test item was instilled into the left conjunctival sac of the eye of each animal. After application, both animals were examined at approximately 1, 24, 48, 72-hours and 7 and 14 days, and one of the animals was also examined at approximately 21 days to verify the presence of lesions in the cornea, iris, eyelid and eyeball conjunctivae, and for behavioural and clinical alterations. The untreated right eye was used as a negative control.

The test item applied in the eye of the rabbits produced corneal opacity, iritis (circumcorneal injection), conjunctival hyperaemia, oedema and secretion in both tested eyes. Corneal opacity and conjunctival hyperaemia were still noted at the end of the observation period in one of two tested eyes. All irritation signs had returned to normal by the 14-day time point following treatment to one of two tested eyes. Fluorescein sodium dye detected treatment-related changes of the surface of the cornea in both tested eyes. Additional ocular changes observed included: blepharitis and a small raised-off area on the corneal surface.

The individual mean irritation scores (24, 48 and 72-hours) of the two rabbits were as follows:

- for corneal opacity: 3.33 and 3.67
- for iris lesions: 1.00 and 1.00
- for conjunctival redness: 3.00 and 2.67
- for chemosis of the conjunctiva: 2.00 and 1.33

Based on the study, glyphosate TC has the potential to seriously damage the eyes under the test conditions chosen.

Materials and methods

A. Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05 %
pH:	2.20 (at 1 %, 23.1 °C)
Stability of test compound:	No data given in the report.

2. Vehicle and/or positive control: No vehicle was used

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	18 weeks old
Sex:	Male and female (nulliparous and non-pregnant)

Weight at dosing:	Male: 3.346; female: 3.624 kg	
Acclimation period:	5 – 6 days	
Diet/Food:	Pelleted and autoclaved commercial diet for rabbits (Guabi, Mogiana Alimentos S.A. - Brazil), *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually in galvanised steel cages. Autoclaved wood shavings were placed in a tray below the cages to collect excrements.	
Environmental conditions:	Temperature:	17 – 22 °C
	Humidity:	30 – 70 %
	Air changes:	10 – 15 / hour
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2008-05-26 to 2008-06-17

Animal assignment and treatment:

One tenth (0.1) g of the test item was applied to the eye of each animal. The test item was applied into the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. Following application, the eyelids were gently held together for about one second in order to prevent test item loss. The right eye remained untreated and was used as a negative control.

The test was performed initially using one animal for evaluation of any irritant / corrosive effect of the test item to the eye. Because some severe ocular reactions were observed in the initial test, only one additional animal was tested to confirm the response.

The animals' eyes were grossly examined with the aid of an auxiliary light source for signs of irritation in the cornea, iris, eyelid and eyeball conjunctivae at approximately 1, 24, 48, 72-hours and 7 and 14 days after test item application to the first rabbit, and at approximately 1, 24, 48, 72-hours and 7, 14 and 21 days after test item application to the second rabbit. The grading of the ocular reactions was done according to the Draize scheme. After recording the observations at 24-hour time point for each animal, the corneal surface on all test and control eyes was examined using a fluorescein sodium dye to detect abnormalities which are not grossly observable. The fluorescein examinations were conducted to each subsequent interval until two negative responses were obtained. In addition, a clinical examination was accomplished to verify the presence or absence of any local or systemic toxic effects.

Results

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No data on clinical signs reported.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No data on necropsy were reported.

E. EYE OBSERVATIONS

Corneal opacity grade 1 – 4, with affected area varying from 1 – 3, was noted at all observation time points and persisted until the end of the observation period for rabbit #05.

Iritis grade 1 (circumcorneal injection) was noted from the 1 – 72-hour time points for both tested rabbits.

Conjunctival effects included: hyperaemia grade 1 – 3, from the 1-hour to the 7-day time points for rabbit #04 and persisted until day 21 in rabbit #05.

Edema grade of 1 – 3 were observed from the 1-hour to the 14-day time points.
For secretion, grades 1 – 2 were noted until the 72-hour time point for one rabbit and until the 14-day time point for the second rabbit.

All eye irritation signs had returned to normal by the 14-day time point for rabbit #04.

Fluorescein sodium dye detected treatment-related changes to the surface of the cornea at the 24 – 72-hour time points for rabbit #05, and at the 24-hour time point for rabbit #04. Blepharitis was observed in the initial treated animal at the 48- and 72-hour time points and in the subsequently treated animal at the 24-, 48- and 72-hour and 7- and 14-day time points. In the second animal a small raised off area on the corneal surface in the right inferior quadrant at the 21-day time point was noted.

Individual mean scores over 24, 48 and 72-hours for each animal were 3.33 and 3.67 for corneal opacity, 1.00 and 1.00 for iris lesions, 1.33 and 2.00 for chemosis as well as 3.00 and 2.67 for redness of the conjunctiva.

[redacted] Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical. [redacted] 2008): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Hyperaemia	Oedema	Secretion
Rabbit 04 (male)	1	1	2	1*	2	3	2
	24	**3**	2	**1***	**3**	**3**	2
	48	**3**	2	**1***	**3**	**2**	2
	72	**4**	1	**1***	**3**	**1**	1
	Day 7	0	0	0	1	1	0
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**3.33**	**---**	**1.00**	**3.00**	**2.00**	**---**
Rabbit 05 (female)	1	3	2	1*	2	1	2
	24	**4**	2	**1***	**2**	**2**	2
	48	**3**	3	**1***	**3**	**1**	2
	72	**4**	3	**1***	**3**	**1**	2
	Day 7	4	2	0	2	1	2
	Day 14	4	2	0	2	1	1
	Day 21	4	1	0	1	0	0
Individual mean (24, 48, 72 h)		**3.67**	**---**	**1.00**	**2.67**	**1.33**	**---**

* Circumcorneal injection

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate technical into the rabbit eye elicited strong response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were 3.33 and 3.67 for corneal opacity, 1.00 and 1.00 for

iris lesions, 1.33 and 2.00 for chemosis, as well as 3.00 and 2.67 for redness of the conjunctiva. These effects were not reversible within 21 days.
Thus, glyphosate is considered to have the potential to seriously damage the eye.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions the test substance is damaging to the rabbit eye. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.5.8. Study 8

Data point:	CA 5.2.5/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits
Report No	B02788
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), 2004/73/EC B.5 (2004), JMAFF 12 NohSan No. 8147 (2000)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, animals were previously used in a primary skin irritation study. This deviation is not considered to affect the study outcome.
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

In a primary eye irritation study, 0.1 g of glyphosate technical (Batch: 0507, Purity: 96.1 %) was instilled into the conjunctival sac of the left eye of each of three young adult New Zealand albino rabbits (one male and two females). The animals were observed for seven days. The ocular reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72-hours as well as 7 days after instillation.

The instillation of glyphosate technical into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae and sclera, discharge and chemosis. These effects were reversible and were no longer evident seven days after treatment. No abnormal findings were observed in the cornea or iris of any animal at any of the examinations. No corrosion was observed at any of the measuring intervals. No staining of the treated eyes by the test item was observed and no clinical signs were observed.

The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:
- for corneal opacity: 0.00, 0.00, 00.0
- for iris lesions: 0.00, 0.00, 0.00
- for conjunctival redness: 0.67, 1.67, 1.67
- for conjunctival chemosis: 0.00, 0.33, 1.00

Based on the study, glyphosate technical was not irritating to the eyes under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical Material
Description:	White powder
Lot/Batch number:	0507
Purity:	96.1 % w/w glyphosate acid
Stability of test compound:	Stable under storage conditions at room temperature (range of 20 ± 5 °C), protected from light and humidity.

2. **Vehicle and/ or positive control:** None

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White (SPF)
Source:	[redacted]
Age:	Male: 11 – 12 weeks: females: 14 – 16 weeks
Sex:	Male and females
Weight at Dosing:	Male: 2640 g; females: 2990 – 3001 g
Acclimation period:	5/6 days (the animals were previously used in the Primary Skin Irritation Study RCC B02777)
Diet:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages equipped with feed hoppers and drinking water bowls. Woodblocks and haysticks provided for gnawing
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: 10 – 15 per hour Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In-life dates: 2006-12-27 to 2007-01-04

Animal assignment and treatment:
On the day of treatment, 0.1 g of the test item was placed into the conjunctival sac of the left eye of each animal after gently pulling the lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of the test substance. The right eye remained untreated and acted as the reference control. The treated eyes were not rinsed after instillation of the test substance.

As it was suspected that the test substance might produce irritancy, a single female was treated first. As neither a corrosive effect nor a severe irritant effect was observed after 1- and 24- hour examinations, the test was completed using the two remaining animals.

The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004, at approximately 1, 24, 48 and 72-hours, as well as 7 days after instillation. Additionally, ocular discharge, reddening of the sclera and staining of conjunctivae, sclera, and cornea by the test substance was assessed according to the scheme presented in the guideline.

The animals were observed daily throughout the study for viability, mortality and clinical signs. Body weights were measured at the start of acclimatisation, on the day of treatment and at termination of the observation period. No necropsy was performed at termination of the study.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed in the animals during the study

C. BODY WEIGHT

The body weights of all rabbits were considered to be within the normal range of variability.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

No abnormal findings were observed in the cornea or iris of any animal at any of the measurement intervals. Moderate reddening of the conjunctivae was noted in all animals at the 1-hour reading and persisted in one animal as slight until the 48-hour reading and in two animals as moderate to slight until 72-hours after treatment. Slight to obvious swelling (chemosis) with partial eversion of the lids was observed in all three animals at the 1-hour reading and persisted with the same severity in two animals at the 24-hours and in one animal as slight until the 48-hour reading. Slight to moderate reddening of the sclera was noted in all animals at the 1- and 24-hour reading and persisted as slight reddening in two animals until the 48-hour reading. Slight to moderate ocular discharge was seen in all animals at the 1-hour reading and persisted as slight to moderate discharge in two animals at the 24-hour reading. No abnormal findings were observed in the treated eye of any animal 7 days after treatment, the end of the observation period for all animals. No staining of the treated eyes produced by the test substance was observed and no corrosion of the cornea was observed at any of the reading times.

The individual mean irritation scores (24 – 72-hours) were calculated to be 0.00 for all animals for corneal opacity and iris lesions, 0.67, 1.67, 1.67 for conjunctival redness, and 0.00, 0.33, 1.00 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 – 72-hours) are presented in the table below.

Table 6.2.5.2-1 Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits. ([redacted] 2007): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva			Sclera
		Opacity	Area		Redness	Chemosis	Discharge	
Rabbit 31 (male)	1	0	0	0	2	1	1	2
	24	**0**	0	**0**	**1**	**0**	0	1
	48	**0**	0	**0**	**1**	**0**	0	0
	72	**0**	0	**0**	**0**	**0**	0	0
	Day 7	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**0.67**	**0.00**	**---**	**---**
Rabbit 32 (female)	1	0	0	0	2	2	1	2
	24	**0**	0	**0**	**2**	**1**	1	2
	48	**0**	0	**0**	**2**	**0**	0	1
	72	**0**	0	**0**	**1**	**0**	0	0
	Day 7	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**1.67**	**0.33**	**---**	**---**

Table 6.2.5.2-1 Glyphosate Technical Material: Primary Eye Irritation Study in Rabbits. ([redacted], 2007): Eye irritation – Individual irritation scores

Rabbit 33 (female)	1	0	0	0	2	2	2	2
	24	**0**	0	**0**	**2**	**2**	2	2
	48	**0**	0	**0**	**2**	**1**	0	1
	72	**0**	0	**0**	**1**	**0**	0	0
	Day 7	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**---**	**0.00**	**1.67**	**1.0**	**---**	**---**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and the previously use of the animals in a skin irritation study, the study is in concordance with the current OECD TG 405 (2017). The deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate technical into the rabbit eye elicited slight response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were 0.00 for all animals for corneal opacity and iris lesions, 0.67, 1.67, 1.67 for conjunctival redness, and 0.00, 0.33, 1.00 for conjunctival chemosis.
These effects were reversible within 7 days. Thus, under test conditions of the study, glyphosate technical is considered not irritating to the eye mucosa of rabbits.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions the test substance is not irritating to the rabbit eye. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.5.9. Study 9

Data point:	CA 5.2.5/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits
Report No	B02305
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), Commission Directive 2004/73/EC B.5 (2004), JMAFF guideline 2-1-5 (2005)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Treated eyes were not rinsed after instillation.
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

In an eye irritation study, 0.1 g of the undiluted, solid glyphosate technical (Batch: 200609062, Purity: 95.1 %) was instilled into the left conjunctival sac of three young adult New Zealand albino rabbits in a stepwise manner.

Animals were observed for 14 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72-hours and 7, 10, and 14 days after test item instillation. Application of glyphosate technical (NUP 05068) into the rabbit eye resulted in marked, early onset and transient ocular changes of very slight to slight corneal opacity, slight to marked conjunctival redness, conjunctival chemosis, reddening of the sclera, and discharge. All eye effects were reversible within 10 days after instillation. No abnormal findings were observed in the iris of any animal at any of the examinations. No signs of corrosion or staining were observed in any eye. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.67, 2.00 and 0.67;
- for iris lesions: 0.00, 0.00, 0.00
- for conjunctival redness: 2.67, 2.00, 2.00
- for chemosis of the conjunctiva: 2.00, 2.00, 1.00.

Based on the study, glyphosate TC has the potential for irritating the eye under the test conditions chosen.

I. MATERIAL AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions (20 ± 5 °C), light protected; Expiry date: 2008-09-14

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	[redacted]
Age:	Male: 15 weeks; females: 12 and 15 weeks
Sex:	Male and females
Weight at dosing:	Male: 2.969 kg; females: 2.605 and 3.416 kg
Acclimation period:	At least five days
Diet/Food:	Pelleted standard Provimi Kliba 3418 rabbit maintenance diet (Provimi Kliba AG, CH-Kaiseraugust, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks and haysticks were provided for gnawing.
Environmental conditions:	Temperature: 17 – 23 °C Humidity: 30 – 70 % Air changes: 10 – 15 / hour Photoperiod: 12 hours light / dark cycle

B. STUDY DESIGN AND METHODS

In life dates: 2007-01-22 to 2007-02-26 (Start of treatment to study completion)

Animal assignment and treatment:
The test was conducted using young adult New Zealand albino rabbits (one male, two females). The test was performed in a sequential manner, first using one animal. Since no corrosive or severe eye effects were observed in the first animal the test was completed using the remaining two rabbits. An amount of 0.1 g of the solid test substance was applied into the conjunctival sac of the left eye of the rabbits. The lids were then gently held together for about one second. The treated eyes were not rinsed after instillation. The right eye remained untreated and served as the reference control. Eye reactions were assessed according to the scoring system listed in Commission Directive 2004/73/EC approximately 1, 24, 48 and 72-hours, as well as 7, 10 and 14 days after instillation. Scleral reddening and ocular discharge were also assessed. Eye examinations were made using a diagnostic lamp. The animals were observed for mortality and clinical signs daily. Body weights were determined at beginning of acclimatisation, on the day of application and at termination.

Numerical scoring system (Draize scale for scoring ocular irritation):

Cornea	
Opacity: degree of density (readings should be taken from most dense area)*	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Area of cornea involved	
Zero	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection (any of these or combination or any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2
Conjunctivae, Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
Blood vessels normal	0
Some blood vessels definitely hyperaemic (injected)	1
Diffuse, crimson colour, individual vessels not easily discernible	2
Diffuse, beefy red	3
Conjunctivae, Chemosis (lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal (including nictitating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half-closed	3
Swelling with lids more than half-closed	4
Discharge	
No discharge	0
Slight: Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Moderate: Discharge with moistening of the lids and hairs just adjacent to lids	2
Marked: Discharge with moistening of the lids and hairs and considerable area around the eye	3

* The area of corneal opacity should be noted

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Very slight to slight corneal opacity was observed in all rabbits from 1 hour after instillation up to 72-hours. No signs of iritis, corrosion, or staining were observed in any animal throughout the study period. One hour after instillation slight to moderate conjunctival redness was observed in the treated eyes of all rabbits. By 24-hours the redness increased to marked in two animals and to moderate in one rabbit and were still persistent at the 48-hour evaluation time point. At 72-hours slight or moderate reddening of the conjunctivae was seen, and a slight reddening persisted in all three animals until Day 7. Moderate to marked chemosis of the conjunctivae was observed from 1 hour after instillation up to 24-hours. The swelling decreased with time. Seventy-two hours after treatment slight swelling was still present in two animals. Moderate ocular discharge was noted in two rabbits one hour after instillation and moderate or marked discharge in all animals at the 24-hour reading time point, which persisted at the 48-hour reading as slight or moderate in all rabbits. After 72-hour slight discharge was still present in one rabbit.

Reddening of the sclera was observed in all animals. However, one hour after instillation sclera of one animal was not assessable due to conjunctival swelling. In two animals moderate or marked reddening of the sclera was observed at this time point. After 24-hours all rabbits showed marked reddening of the sclera. This sign persisted in the rabbits as moderate or marked at the 48- and 72-hour readings. In one animal slight reddening was still present after 7 days.

All rabbits were free of ocular signs by Day 10 after instillation.

Individual mean scores calculated for the three animals over 24, 48 and 72-hours were 1.67, 2.00 and 0.67 for corneal opacity, 0.00, 0.00 and 0.00 for iris lesions, 2.67, 2.00 and 2.00 for redness of the conjunctiva, and 2.00, 2.00 and 1.0 for chemosis, respectively.

Table 6.2.5.3-1 Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits. [redacted] 2007): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva			Sclera
		Opacity	Area		Redness	Chemosis	Discharge	
Rabbit 10 (male)	1	1	1	0	2	3	2	3
	24	**2**	2	**0**	**3**	**3**	2	3
	48	**2**	2	**0**	**3**	**2**	1	3
	72	**1**	1	**0**	**2**	**1**	0	2
	Day 7	0	0	0	1	0	0	1
	Day 10	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.67**	**---**	**0.0**	**2.67**	**2.00**	**---**	**---**
Rabbit 11 (female)	1	1	1	0	1	2	0	2
	24	**2**	4	**0**	**2**	**3**	3	3
	48	**2**	4	**0**	**2**	**2**	2	3
	72	**2**	4	**0**	**2**	**1**	1	2
	Day 7	0	0	0	1	0	0	0

Table 6.2.5.3-1 Glyphosate Technical (NUP 05068): Primary Eye Irritation Study in Rabbits. ([redacted] 2007): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva			Sclera
		Opacity	Area		Redness	Chemosis	Discharge	
	Day 10	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**0.0**	**2.00**	**2.00**	---	**---**
Rabbit 12 (female)	1	1	4	0	2	3	2	n.a.
	24	**1**	4	**0**	**3**	**2**	2	3
	48	**1**	4	**0**	**2**	**1**	1	2
	72	**0**	0	**0**	**1**	**0**	0	2
	Day 7	0	0	0	1	0	0	0
	Day 10	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.67**	**---**	**0.0**	**2.00**	**1.00**	---	**---**

n.a. = not assessable due to swelling of the conjunctivae

3. Assessment and conclusion

Assessment and conclusion by applicant:
Compared to the current OECD TG 405 (2017), minor deviations such as no treatment with systemic analgesic or topical anaesthesia of the animals, no rinsing after test substance instillation are noted.
As the lack of rinsing is considered as worst-case, this deviation is not considered to underestimate the results the study outcome. Therefore, the study is considered acceptable and the outcome can be reported as valid.
A single instillation of glyphosate into the rabbit eye revealed marked, early onset and transient ocular changes, such as corneal opacity, reddening of the conjunctivae and sclera, discharge and chemosis. All eye effects were reversible within 10 days after instillation. The mean individual score over 24, 48 and 72-hours was 1.67, 2.00 and 0.67 for corneal opacity, 0.00, 0.00 and 0.00 for iris lesions, 2.67, 2.00 and 2.00 for redness of the conjunctiva, and 2.00, 2.00 and 1.00 for chemosis.
Thus, based on the study results, the test substance glyphosate technical (NUP 05068) is considered to be irritating to the rabbit eye. Nevertheless, for classification purposes the serious eye damage observed in another study should be taken into account.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions the test substance is irritating to the rabbit eye. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.5.10. Study 10

Data point:	CA 5.2.5/010
Report author	[redacted]
Report year	2005
Report title	Glyphosate Acid Technical - Primary Eye Irritation Study in Rabbits
Report No	15277
Document No	Not reported
Guidelines followed in study	OECD 405 (2002), US EPA OPPTS 870.2400 (1998), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. All three animals were treated at once instead of a stepwise manner. Animal weights and age were not recorded. The amount of test substance tested is low. The

	humidity and number of air changes were 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 but with restrictions (reliable with restrictions) due to the low amount of test substance used.

A primary eye irritation test was conducted with rabbits to determine the potential for glyphosate acid technical (Batch: 040205, Purity: 97.23 %) to produce irritation from a single instillation via the ocular route. Prior to use, the test substance was ground to a powder and 0.06 g of the ground test substance was instilled into the right eye of three healthy rabbits. The left eye remained and served as control. Animals were observed for 10 days. Ocular irritation was evaluated by the Draize scheme 1, 24, 48 and 72-hours and 4, 7 and 10 days after test item instillation. Application of glyphosate acid technical into the rabbit eye elicited corneal opacity, iritis, and conjunctivitis one hour after test substance instillation in all three animals. The overall incidence and severity of irritation decreased gradually over time. All eye irritation effects were fully reversible by Day 10.

The individual mean irritation scores (24, 48 and 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00 and 1.00
- for iris lesions: 1.00, 1.00 and 1.00
- for conjunctival redness: 2.33, 2.67 and 2.67
- for chemosis of the conjunctiva: 1.67, 2.00 and 2.00.

Based on the study, glyphosate TC was irritating the eye under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 %
Water solubility:	12 g/L
pH:	2.5 in 1 % solution
Stability of test compound:	No data given in the report. Test substance was expected to be stable for the duration of testing.

2. Vehicle and/or positive control: No vehicle was used

3. Test animals:

Species:	Rabbit
Strain:	New Zealand albino
Source:	[redacted]
Age:	Young adult
Sex:	Males
Weight at dosing:	No data given in the report
Acclimation period:	7 days
Diet/Food:	Pelleted Purina Rabbit Chow #5326

Water:	Tap water, *ad libitum*	
Housing:	Individually in suspended stainless steel cages with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.	
Environmental conditions:	Temperature:	18 – 22 °C
	Humidity:	Not reported
	Air changes:	Not reported
	Photoperiod:	12-hour light / dark cycle

B: Study design and methods

In life dates: 2004-05-26 to 2004-06-05

Animal assignment and treatment:

Prior to use, the test substance was ground to a powder. One-tenth of a milliliter (0.06 g) of the ground test substance was instilled into the right eye of three healthy rabbits, which were selected based on fluorescein dye examinations of their eyes prior to instillation of the test substance. The left eye remained untreated and served as control. Ocular irritation was evaluated by the Draize method at 1, 24, 48 and 72-hours as well as at 4, 7 and 10 days post-instillation. The fluorescein dye evaluation procedure was used at 24-hours and as needed at subsequent scoring intervals to evaluate the extent of corneal damage or to verify reversibility of effects. Individual scores were recorded for each animal. In addition to observations of the cornea, iris, and conjunctivae, any other observed lesions were noted. The average individual score for all rabbits at 24, 48 and 72-hours was calculated. Additionally, animals were observed for signs of gross toxicity and behavioural changes at least once daily during the test period. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somato-motor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea and coma.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

There were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour.

C. BODY WEIGHT

Body weight not reported.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Results of the eye irritation assessment are presented in the table below. One hour after test substance instillation, all three treated eyes exhibited corneal opacity, iritis, and conjunctivitis. The overall incidence and severity of irritation decreased gradually over time. All animals were free of ocular irritation by Day 10 (study termination).

Individual mean scores over 24, 48 and 72-hours for each animal were 1.00 for corneal opacity and iris lesions, 2.33, 2.67 and 2.67 for redness of the conjunctiva, and 1.67, 2.00 and 2.00 for chemosis.

Table 6.2.5.4-1 Primary Eye Irritation Study in Rabbits. [redacted] 2005): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 11915 (male)	1	1	1	1	3	2	2
	24	**1[1]**	4	**1**	**3**	**2**	2[2]
	48	**1**	4	**1**	**2**	**2**	2[2]
	72	**1[1]**	4	**1**	**2**	**1**	2
	Day 4	1[1]	4	1	2	1	1
	Day 7	1[1]	2	0	1	0	0
	Day 10	0[1]	4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.33**	**1.67**	**---**
Rabbit 11916 (male)	1	1	1	1	3	2	2
	24	**1[1]**	4	**1**	**3**	**3**	2
	48	**1**	4	**1**	**3**	**2**	2
	72	**1[1]**	4	**1**	**2**	**1**	2
	Day 4	1[1]	4	1	2	1	1
	Day 7	1[1]	2	0	2	0	0
	Day 10	0[1]	4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.67**	**2.00**	**---**
Rabbit 11917 (male)	1	1	1	1	2	2	2
	24	**1[1]**	4	**1**	**3**	**3**	2
	48	**1**	4	**1**	**3**	**2**	2
	72	**1[1]**	4	**1**	**2**	**1**	2
	Day 4	1[1]	4	1	2	1	1
	Day 7	1[1]	2	0	1	0	0
	Day 10	0[1]	4	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.67**	**2.00**	**---**

[1] 2 % ophthalmic fluorescein sodium used to evaluate the extent or verify the absence of corneal opacity.
[2] Cream white-coloured discharge noted.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application the study is in concordance with the current OECD TG 405 (2017). These deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Nevertheless, due to the low amount of the test substance (0.06 g), the reliability of the study is considered supplementary, only.
Instillation of glyphosate TC into the rabbit eye elicited positive response in all animals. The individual mean scores over 24, 48 and 72-hours for each animal were 1.00 for all animals for corneal opacity and iris lesions, 2.33, 2.67 and 2.67 for redness of the conjunctiva, and 1.67, 2.00 and 2.00 for chemosis. These effects were fully reversible within 10 days. Thus, glyphosate is considered irritant to the eye.
Nevertheless, for classification purposes all eye irritation studies should be taken into account.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant that the study is considered supplementary due to the low amount of test substance used. Under the study conditions the test substance is considered irritating to the rabbit eye (Category 2), when combining the information on the severity scores and reversibility of the scores.

B.6.2.5.11. Study 11

Data point:	CA 5.2.5/011
Report author	[redacted]
Report year	1997
Report title	Glyphosate Acid: Eye Irritation to the Rabbit
Report No	[redacted] P/5138
Document No	Not reported
Guidelines followed in study	OECD 405 (1987), 92/69/EEC B.5 (1992), EPA 81-4
Deviations from current test guideline (OECD 405, 2017)	Six instead of three animals, no treatment with systemic analgesic prior, during, or after test substance application, only local anaesthetic ophthaine was used in five of six animals prior dosing. The age of the animals was not reported. These deviations are not considered to affect the study outcome.
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

In a primary eye irritation study, 0.1 g of glyphosate acid (Batch: P24, Purity: 95.6 % (w/w)) was instilled into the conjunctival sac of the left eye of one of six young adult New Zealand White albino female rabbits. When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously.

The eyes were examined using the Draize scale to assess the grade of ocular reaction approximately at 1, 24, 48, 72-hour time points and after 4, 7 and 8 days after application. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system was used to interpret and classify the numerical scores. Body weight was determined on the day of dosing.

Corneal, iridial and conjunctival effects were seen in all animals for up to 4 days. All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on Day 7; the animal had completely recovered by Day 8.

The individual mean irritation scores (24 – 72-hours) of the six rabbits were as follows:

- for corneal opacity: 0.67, 1.00, 1.33, 2.00, 1.00, 2.00,
- for iris lesions: 0.33, 0.67, 0.67, 0.67, 1.00, 1.00,
- for conjunctival redness: 1.67, 2.00, 2.00, 2.00, 2.00, 2.00
- for conjunctival chemosis: 1.33, 1.33, 1.67, 2.00, 2.00, 2.00.

Based on the study, glyphosate acid has the potential for irritation of the eyes under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification: Glyphosate acid

Description: White solid

Lot/Batch number:	P24
Purity:	95.6 % (w/w)
CAS#:	Not reported
Stability of test compound:	The test substance was used within the expiry date (expiry date Not reported)

2. **Vehicle and/ or positive control:** None

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White albino
Source:	[redacted]
Age:	Young adult (age Not reported)
Sex:	Females
Weight at dosing:	2951 – 3702 g
Acclimation period:	At least 6 days
Diet:	STANRAB SQC, (Special Diet Services Limited, Stepfield, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium sheet cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Environmental conditions:	Temperature: 17 ± 2 °C Humidity: 40 – 70 % Air changes: Approximately 25 – 30 / hour Photoperiod: 12 hours light / 12 hours dark

B: Study design and methods

In-life dates: 1996-05-22 to 1996-07-06

Animal assignment and treatment:

Initially, the test substance (approximately 0.1 g) was applied into the conjunctival sac of the left eye of one rabbit by gently pulling the lower lid away from the eyeball. The lids were then gently held together for 1 – 2 seconds after which the animal was released. The other eye remained untreated (control eye). When the eye irritation potential had been fully assessed in the first animal, the test substance was applied into the test eye of the remaining five animals, as described previously. As the initial pain reaction of the first rabbit was moderate and the irritation was less than severe, the eyes of the remaining rabbits were pre-treated with five drops of local anaesthetic (Ophthaine, 0.5 % proparacaine hydrochloride solution) with three minute intervals between each drop.

Both eyes of each rabbit were examined within the twenty-four hours prior to dosing. The examination consisted of a visual assessment with the aid of fluorescein and only rabbits without any apparent eye defects or ocular irritation were used. Immediately after the application of the test substance, an assessment of the initial pain reaction of the rabbit was made using a six-point scale.
The eyes were examined and the Draize scale was used to assess the grade of ocular reaction approximately one hour and 1, 2, 3, 4, 7 and 8 days after application where necessary. In addition, as an aid in the assessment of corneal damage, fluorescein staining was used at all readings from 1 day after application. A modified form of the Kay and Calandra system (Kay and Calandra, 1962) was used to interpret and classify the numerical scores.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No systemic signs of toxicity were noted during the study. Application into the eye caused moderate initial pain in the first animal dosed; therefore the subsequent five animals were pre-treated with the local anaesthetic OPHTHAINE prior to dosing. The group initial pain reaction was none to moderate (class 0 – 3 on a 0 – 5 scale).

C. BODY WEIGHT

Not reported.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

Between ¼ and ½ of the test item was displaced in every animal immediately after dosing.

Corneal effects, consisting of slight to mild opacity affecting up to the entire cornea, were seen in all animals during the first two days, persisting to Day 4 in five rabbits. Slight iritis was seen in all animals during the first two days, persisting to Day 3 in two rabbits. Conjunctival effects consisting of slight to moderate redness, slight to mild chemosis, and slight to severe discharge, were seen in all animals up to Day 4.

Additional observations included mucoid discharge, eye closed, irregular corneal surface, convoluted eyelids, and erythema of the upper and/or lower eyelids, raised corneal opacity, Harderian gland discharge and nictitating membrane partially haemorrhagic.

All signs of irritation had completely regressed in five animals 7 days after application. Slight conjunctival redness was seen in the remaining animal on Day 7; the animal had completely recovered by Day 8.

The individual mean irritation scores (24 – 72-hours) were calculated to be 0.67, 1.00, 1.33, 2.00, 1.00, 2.00 for corneal opacity, 0.33, 0.67, 0.67, 0.67, 1.00, 1.00 for iris lesions, 1.67, 2.00, 2.00, 2.00, 2.00, 2.00 for conjunctival redness and 1.33, 1.33, 1.67, 2.00, 2.00, 2.00 for conjunctival chemosis. The individual scores for each time point, individual mean scores (24 – 72-hours) are presented in the table below.

Table 6.2.5.5-1 Glyphosate Acid: Eye Irritation to the Rabbit. [redacted] 1997): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 17 (female)	1	0	0	0	1	2	2
	24	**1**	1	**0**	**2**	**2**	3
	48	**1**	1	**1**	**2**	**1**	3
	72	**0**	0	**0**	**1**	**1**	2
	Day 4	0	0	0	1	0	1
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.67**	**---**	**0.33**	**1.67**	**1.33**	**---**
Rabbit 18 (female)	1	0	0	0	2	2	1
	24	**1**	4	**1**	**2**	**2**	3
	48	**1**	4	**1**	**2**	**1**	3
	72	**1**	3	**0**	**2**	**1**	1

Table 6.2.5.5-1 Glyphosate Acid: Eye Irritation to the Rabbit. [redacted] 1997): Eye irritation – Individual irritation scores

	Day 4	1	1	0	1	1	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**0.67**	**2.00**	**1.33**	**---**
Rabbit 19 (female)	1	0	0	0	2	1	1
	24	**2**	4	**1**	**2**	**2**	3
	48	**1**	4	**1**	**2**	**2**	3
	72	**1**	2	**0**	**2**	**1**	1
	Day 4	1	1	0	1	1	0
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.33**	**---**	**0.67**	**2.00**	**1.67**	**---**
Rabbit 7 (female)	1	1	1	0	1	1	2
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	4	**0**	**2**	**2**	3
	Day 4	1	3	0	2	1	1
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**0.67**	**2.00**	**2.00**	**---**
Rabbit 8 (female)	1	1	1	0	2	1	2
	24	**1**	2	**1**	**2**	**2**	2
	48	**1**	2	**1**	**2**	**2**	2
	72	**1**	2	**1**	**2**	**2**	2
	Day 4	1	2	0	2	1	1
	Day 7	0	0	0	1	0	0
	Day 8	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**---**	**1.00**	**2.00**	**2.00**	**---**
Rabbit 9 (female)	1	1	1	0	2	1	2
	24	**2**	4	**1**	**2**	**2**	2
	48	**2**	4	**1**	**2**	**2**	2
	72	**2**	3	**1**	**2**	**2**	2
	Day 4	1	3	0	2	1	1
	Day 7	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**2.00**	**---**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and the use of six instead of three animals the study is in concordance with the current OECD TG 405 (2017). This deviation did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate acid into the rabbit eye elicited moderate to strong response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were 0.67, 1.00, 1.33, 2.00, 1.00, 2.00 for corneal opacity, 0.33, 0.67, 0.67, 0.67, 1.00, 1.00 for iris lesions, 1.67, 2.00, 2.00, 2.00, 2.00, 2.00 for conjunctival redness and 1.33, 1.33, 1.67, 2.00, 2.00, 20.0 for conjunctival chemosis.

The effects were reversible within 8 days. Thus, glyphosate acid is considered irritating to rabbit eye.

Assessment and conclusion by RMS:

Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions the test substance is irritating to the rabbit eye. This conclusion is in line with the previous assessment (RAR, 2015).

B.6.2.5.12. Study 12

Data point:	CA 5.2.5/012
Report author	[redacted]
Report year	1996
Report title	Primary eye irritation study in rabbits
Report No	2981-96
Document No	
Guidelines followed in study	US EPA 81-4 (1984)
Deviations from current test guideline (OECD 405 (2017))	No treatment with systemic analgesic or topical anaesthesia of the animals/animal eyes prior, during or after test substance application. Nine animals treated at the same time, and no tiered testing approach was used. A washout was performed after 30 seconds (in three animals) instead of 1 hour. Animal age is not specified. The humidity was outside the range specified by the OECD guideline.
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, although some eyes were rinsed prior to 1 hour of exposure the exposure time was sufficient to show eye damage.

In an eye irritation study, a 0.1 mL volume (equivalent to 0.065 g) of the undiluted glyphosate technical (Batch: 120594, Purity: 98.2 %) was instilled into the right conjunctival sac of nine New Zealand White rabbits. For three animals, the eyes were washed out for one minute with deionised water 30 seconds after treatment with the test item. The eyes of the remaining six animals were washed out at 24-hours after treatment. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48, 72-hours, Day 4, 7, 10, 14, 17 and 21. A fluorescein staining was performed at 24-hours and repeated at each observation time point until staining was no longer observed.

Application of glyphosate technical into the rabbit eye resulted in corneal opacity and effects on the conjunctivae, which all resolved after until Day 17, except for two of six eyes (without washing) in which corneal opacity, conjunctival discharge and chemosis persisted until termination of the study. As the washing step was performed too early, the results of these animals will not be shown or discussed any further in the following. The individual mean irritation scores (24 – 72-hours) of eyes that were washed at 24-hours were as follows:

- for corneal opacity: 1.00, 1.00, 2.00, 1.00 1.00, 1.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 2.67, 3.00, 2.67, 3.00, 2.33, 3.00
- for chemosis of the conjunctiva: 1.67, 2.67, 2.33, 2.00, 2.00, 2.33

Based on the study, glyphosate has the potential to causes serious eye damage under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification: Glyphosate Technical (WetCake)

Description:	White powder
Lot/Batch #:	120594
Purity:	98.2 %
Stability of test compound:	Expiry date: 1997-09-08

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Male and Female
Initial body weight:	Males: 2.600 – 3.150 kg; females: 2.450 – 2.875 kg
Acclimation period:	5 days
Diet/Food:	PMI feeds lab rabbit chow #5321 or #5326, in measured amounts
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 22 ± 3 °C
	Humidity: 30 – 80 %
	Air changes: 10 – 12 / hour
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1996-07-15 to 1996-08-05

Animal assignment and treatment:

Six male and three female rabbits were prepared for the study. Both eyes of all animals were examined with a fluorescein sodium ophthalmic solution 24-hours before administration.

A dose of 0.1 mL by volume (equivalent to 0.065 g) of glyphosate technical was placed into the right conjunctival sac of one eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. After 30 seconds, the eyes of three male animals were washed out for one minute with deionized water. The results of the animals with the washing step are not discussed below.

At 1, 24, 48 and 72-hours and at Day 4, 7, 10, 14, 17 and 21, all eyes were examined for signs of irritation under normal lighting without magnification. In addition, the eyes were examined after 24-hours with a fluorescein sodium ophthalmic solution. This procedure was repeated at every observation time point until fluorescein staining was no longer present. After the 24-hour recording, all eyes were washed out with deionised water for one minute. Effects on cornea, iris, and conjunctivae (redness and chemosis) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

All rabbits that were washed at 24-hours showed signs of corneal opacity, which persisted in two of six animals until the end of the study. None of the animals showed iridial reactions. Conjunctival redness was observed in all animals. It persisted in two of six animals until the end of the study. In addition, conjunctival chemosis and discharge were observed in all animals. However, they recovered within 17 days, except for one of six animals. Fluorescein staining was observed in all animals at 24-hours and was not observed in any animal by Day 21.

A summary of the findings is given in the table below.

Table 6.2.5.6-1 Primary eye irritation study in rabbits [redacted] 1996): Eye irritation – Individual irritation scores for animals without wash step

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
3412-M (male)	1	0	0	0	2	1	2
	24	**1**	1	**0**	**3**	**2**	0
	48	**1**	1	**0**	**3**	**2**	0
	72	**1**	1	**0**	**2**	**1**	0
	Day 4	0	0	0	2	1	0
	Day 7	0	0	0	1	0	0
	Day 10	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0
	Day 17	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**2.67**	**1.67**	-
3414-M (male)	1	1	1	0	2	2	2
	24	**1**	4	0	**3**	**3**	2
	48	**1**	4	0	**3**	**3**	3
	72	**1**	4	0	**3**	**2**	2
	Day 4	1	2	0	2	1	1
	Day 7	1	1	0	2	0	1
	Day 10	1	1	0	2	0	1
	Day 14	0	0	0	1	0	0
	Day 17	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**3.00**	**2.67**	-
3416-M (male)	1	1	2	0	2	2	2
	24	**2**	1	0	**3**	**3**	1
	48	**2**	1	0	**3**	**2**	1
	72	**2**	1	0	**2**	**2**	1
	Day 4	2	2	0	2	2	1
	Day 7	2	3	0	2	1	1
	Day 10	2	2	0	2	1	1
	Day 14	1	2	0	1	1	1
	Day 17	1	1	0	1	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**-**	**0.00**	**2.67**	**2.33**	-
3411-F (female)	1	1	1	0	2	2	2

Table 6.2.5.6-1 Primary eye irritation study in rabbits [redacted] 1996): Eye irritation – Individual irritation scores for animals without wash step

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	24	**1**	4	0	**3**	**2**	1
	48	**1**	4	0	**3**	**2**	1
	72	**1**	4	0	**3**	**2**	1
	Day 4	1	4	0	3	2	1
	Day 7	1	4	0	3	2	0
	Day 10	1	3	0	2	2	0
	Day 14	2	1	0	1	1	0
	Day 17	2	1	0	1	1	0
	Day 21	2	1	0	1	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**3.00**	**2.00**	-
3413-F (female)	1	1	2	0	2	2	2
	24	**1**	4	0	**3**	**2**	2
	48	**1**	4	0	**2**	**2**	2
	72	**1**	4	0	**2**	**2**	2
	Day 4	1	3	0	2	0	0
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 14	1	1	0	1	0	0
	Day 17	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**2.33**	**2.00**	-
3415-F (female)	1	1	2	0	2	2	2
	24	**1**	4	0	**3**	**3**	2
	48	**1**	4	0	**3**	**2**	2
	72	**1**	4	0	**3**	**2**	1
	Day 4	1	2	0	2	1	1
	Day 7	1	2	0	2	0	1
	Day 10	1	2	0	2	0	1
	Day 14	1	3	0	3	2	2
	Day 17	1	4	0	3	2	1
	Day 21	2	1	0	2	1	0
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.00**	**3.00**	**2.33**	-

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate into the rabbit eye elicited a moderate response in the treated animal. The individual mean scores at 24, 48 and 72-hours for the animals were 1.00, 1.00, 2.00, 1.00, 1.00, 1.00 for corneal opacity, 0.00 for all animals for iris lesions, 2.67, 3.00, 2.67, 3.00, 2.33, 3.00 for conjunctival redness, and 1.67, 2.67, 2.33, 2.00, 2.00, 2.33 for conjunctival chemosis.
Thus, under test conditions of the study, glyphosate has the potential to cause serious eye damage.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions

the test substance causes irreversible eye damage in at least 2 animals up to day 21.

B.6.2.5.13. Study 13

Data point:	CA 5.2.5/013
Report author	[redacted]
Report year	1995
Report title	HR-001: Primary Eye Irritation study in rabbits
Report No	[redacted]95-0034
Document No	Not reported
Guidelines followed in study	OECD 405 (1987), US EPA FIFRA Guideline Subdivision F (1984), JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 405, 2017)	Number of animals (12), no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Testing of further animals (groups B and C) after irritating effects observed in group A. Irrigation after 30 seconds and 2 minutes in testing groups B and C, no irrigation in group A. According to the OECD test guideline washing is allowed after 1 hour post dosing for solids. Therefore the results for group B and C are not guideline compliant. The stability of the test chemical is 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, although some eyes were rinsed prior to 1 hour of exposure the exposure time was sufficient to show eye damage.

In an eye irritation study, HR-001 (Batch: T-941209, Purity: 97.56 %) was applied to the eye of 12 female specific pathogen free New Zealand White rabbits to evaluate its primary eye irritating potential. This study was performed according to the method of Draize. Firstly, six animals were assigned to Group A (without eye irrigation after application). Secondly, three animals each were assigned to Group B (with eye irrigation at 30 seconds after application) and Group C (with eye irrigation at 2 minutes after application), since eye irritation was observed in Group A.

- Irritation of cornea: One hour after application, all animals of Group A showed a score of 2. Twenty-four hours after application, one animal of Group A showed a score of 3. At 1 or 24-hours after application, all animals of Group B and one animal of Group C showed a score of 1. As for the area of cornea involved, Group A showed a score of 4, while Groups B and C showed scores of 2. These opacities disappeared by Day 16 in three of six animals of Group A, and the opacity (score of 1) remained until Day 21 in the other three animals of Group A. In Group B, the opacity disappeared by Day 4, and in Group C, 48 hours after application.

- Irritation of iris: One hour after application, all animals of Group A and C showed scores of 1. In Group B, one animal showed a score of 1 at 1 hour, and one animal showed a score of 1 at 24-hours after application. The irritation of iris disappeared by Day 10 in Group A, 48 hours after application in Group B and 24-hours after application in Group C.

- Irritation of conjunctivae: One hour after application, all groups showed redness scores of 1 and chemosis scores of 2. In addition, some animals in Groups A and B showed chemosis scores of 3. Groups B and C showed discharge scores of 2, and Groups A and C showed discharge scores of 3. Twenty-four or 48 hours after application, scores of 1 for redness of conjunctivae in all animals of Group A, two animals in Group B, and one animal in Group C had changed to scores of 2. These

conjunctival irritations gradually began to decrease thereafter and disappeared by Day 16 in Group A and by Day 7 in Groups B and C.

- Corneal vascularisation: On Day 10, slight corneal vascularisation was observed in three animals of Group A. This sign remained until Day 21 after application.

Without irrigation, the individual mean scores over 24, 48 and 72-hours for each animal were as follows:
- for corneal opacity: 2.00, 2.67. 2.00, 2.00, 2.00, 1.67
- for iris lesions: 1.00, 1.00, 1.00, 1.00, 1.00, 0.67
- for conjunctival redness: 2.00 for all animals
- for conjunctival chemosis: 2.00, 1.67, 2.33, 2.33, 2.00, 1.67

The effects were not reversible within 21 days.

Based on the study, glyphosate technical caused serious eye damage under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	HR-001 (glyphosate technical)
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Water solubility:	12 g/L (25 °C)
Stability of test compound:	Not reported

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, Kbl:NZW
Source:	[redacted]
Age:	11 weeks
Sex:	Females
Weight at dosing:	2237 – 2570 g
Acclimation period:	11 days
Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd., Tokyo, Japan), *ad libitum*
Water:	Water filtered and sterilized, *ad libitum*
Housing:	Individually in stainless steel cages
Environmental conditions:	Temperature: 23.9 – 24.0 °C
	Humidity: 52.8 – 57.9 %
	Air changes: 15 times per hour
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1995-05-09 – 1995-05-30 (Start of treatment to study completion)

Animal assignment and treatment:
Twelve female specific pathogen free New Zealand rabbits were given a single ocular instillation of 0.1 g of technical glyphosate. The dose was instilled in the conjunctival sac of the left eye of each animal after gently

pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to prevent loss of test substance. The treated left eyes of animals in Groups B and C were irrigated with lukewarm water 30 seconds (Group B, three animals) or 2 minutes (Group C, three animals) after application. The right eye remained untreated. No irrigation took place in Group A (six animals). All animals were observed for primary eye irritation 1, 24, 48 and 72-hours, 4 and 7 days after application of the test substance, whereas animals of Group A were also observed at 10, 13, 16, 19 and 21 days after instillation. The cornea, iris and conjunctive were examined with a hand slit-lamp during the observation period and findings were scored according to the criteria described in the guideline of MAFF in Japan and the method of Draize. Body weights were measured prior to application, and after the final observation.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

All rabbits showed the expected body weight gain.

D. NECROPSY

No necropsy was performed.

E. EYE OBSERVATIONS

Results of the group without irrigating are summarised in the table below.

Without eye irrigation

- **Irritation of cornea**

One hour after application, all animals showed score 2 (easily discernible translucent area, details of iris slightly obscured). Twenty-four hours after application, one animal showed score 3 (nacreous area, no details of iris visible, size of pupil barely discernible). Concerning the area of cornea involved, Group A animals showed score 4 (greater than three quarters, up to the whole area of the cornea). These opacities disappeared by Day 16 in three of six animals and a score of 1 remained until Day 21 in the other three animals. On Day 10, slight corneal vascularization was observed in three animals; this sign remained until Day 21.

- **Irritation of iris**

One hour after application, all animals showed score 1 (congestion and / or markedly deepened rugae of iris). The irritation disappeared by Day 10.

- **Irritation of conjunctivae**

Redness: One hour after application, all animals showed redness score 1 (definite hyperaemia of some blood vessels). Twenty-four and 48 hours after application, score 2 (redness of conjunctivae) was observed in all animals. The redness disappeared by Day 16.
Chemosis: One hour after application, four animals showed chemosis score 2 (obvious swelling with partial eversion of lids about half closed). In addition, 2 animals showed score 3 (swelling with lids more than half closed). The chemosis disappeared by Day 7.

Discharge: One hour after application all tested animals showed score 3 (discharge with moistening of the lids and hairs just adjacent around to lids). The discharge disappeared by Day 7.

All these conjunctival irritations gradually weakened and disappeared by Day 16.

The individual mean scores over 24, 48 and 72-hours for each animal were: 2.00, 2.67, 2.00, 2.00, 2.00 and 1.67 for corneal opacity; 1.00, 1.00, 1.00, 1.00, 1.00 and 0.67 for iris lesions; 2.00 for all animals for conjunctival redness; and 2.00, 1.67, 2.33, 2.33, 2.00 and 1.67 for conjunctival chemosis. The effects were not reversible within 21 days. Thus, glyphosate technical is considered severely irritating to the eye mucosa of rabbits.

With eye irrigation (30 seconds or 2 minutes after application)
The iridial and conjunctival irritations observed in the irrigation groups (Groups B and C, see tables below, respectively) were almost the same as those in non-irrigating group (Group A), while the corneal irritation was less pronounced when the test substance was irrigated.

Animals in the irrigating groups showed reduced eye irritations and faster recovery as compared with animals of the non-irrigation group. Irrigation 30 seconds or 2 minutes after application was effective for reduction of the induced irritation and for recovery.

Table 6.2.5.7-1 HR-001: Primary Eye irritation study in rabbits. [redacted] 1995): Eye irritation without eye irrigation after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (female)	1	2	4	1	1	2	3
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	4	**1**	**2**	**2**	1
	Day 4	1	3	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1+	1	0	1	0	0
	Day 13	1+	1	0	1	0	0
	Day 16	1+	1	0	0	0	0
	Day 19	1+	1	0	0	0	0
	Day 21	1+	1	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	---	**1.00**	**2.00**	**2.00**	---
Rabbit 2 (female)	1	2	4	1	1	2	3
	24	**3**	4	**1**	**2**	**2**	3
	48	**3**	4	**1**	**2**	**2**	3
	72	**2**	3	**1**	**2**	**1**	1
	Day 4	2	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1+	2	0	1	0	0
	Day 13	1+	2	0	1	0	0
	Day 16	1+	2	0	0	0	0
	Day 19	1+	2	0	0	0	0
	Day 21	1+	2	0	0	0	0
Individual mean (24, 48, 72 h)		**2.67**	---	**1.00**	**2.00**	**1.67**	---
Rabbit 3 (female)	1	2	4	1	1	3	3
	24	**2**	4	**1**	**2**	**3**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	4	**1**	**2**	**2**	3
	Day 4	2	3	1	2	1	0
	Day 7	1	2	1	1	0	0
	Day 10	1+	2	0	1	0	0

Table 6.2.5.7-1 HR-001: Primary Eye irritation study in rabbits. [redacted] 1995): Eye irritation without eye irrigation after application – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
	Day 13	1+	2	0	1	0	0
	Day 16	1+	2	0	0	0	0
	Day 19	1+	1	0	0	0	0
	Day 21	1+	1	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**2.33**	**---**
Rabbit 4 (female)	1	2	4	1	1	3	3
	24	**2**	4	**1**	**2**	**3**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	3	**1**	**2**	**2**	1
	Day 4	2	2	1	2	1	1
	Day 7	1	2	0	1	0	0
	Day 10	1	2	0	1	0	0
	Day 13	1	2	0	1	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**2.33**	**---**
Rabbit 5 (female)	1	2	4	1	1	2	3
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	4	**1**	**2**	**2**	3
	72	**2**	4	**1**	**2**	**2**	1
	Day 4	1	2	1	2	1	0
	Day 7	1	2	0	1	0	0
	Day 10	1	1	0	1	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	**---**	**1.00**	**2.00**	**2.00**	**---**
Rabbit 6 (female)	1	2	4	1	1	2	3
	24	**2**	4	**1**	**2**	**2**	3
	48	**2**	2	**1**	**2**	**2**	2
	72	**1**	2	**0**	**2**	**1**	0
	Day 4	1	1	0	1	0	0
	Day 7	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0
	Day 13	0	0	0	0	0	0
	Day 16	0	0	0	0	0	0
	Day 19	0	0	0	0	0	0
	Day 21	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.67**	**---**	**0.67**	**2.00**	**1.67**	**---**

+ = slight corneal vascularisation

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the use of 12 instead of 3 animals and no irrigation or after 30

seconds or after 2 minutes instead of 1 hour as recommended for solid test compounds the study follows the current OECD TG 405 (2017). No irrigation is considered to represent a worst-case scenario which did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate technical into the rabbit eye elicited moderate response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were: 2.00, 2.67. 2.00, 2.00, 2.00, 1.67 for corneal opacity; 1.00, 1.00, 1.00, 1.00, 1.00, 0.67 for iris lesions; 2.0 for all animals for conjunctival redness; and 2.00, 1.67, 2.33, 2.33, 2.00, 1.67 for conjunctival chemosis.
The effects were not reversible within 21 days. Thus, under test conditions of the study, glyphosate technical is considered severely irritating to the eye mucosa of rabbits.

Assessment and conclusion by RMS:
Agreed with the conclusion by the applicant. The study is considered acceptable and under the study conditions the test substance causes irreversible eye damage in at least 3 animals up to day 21.

B.6.2.5.14. Study 14

Data point:	CA 5.2.5/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate premix: Acute eye irritation test in the rabbit
Report No	545/41
Document No	Not reported
Guidelines followed in study	US EPA 81-4 (1984), US EPA 798.4500
Deviations from current test guideline (OECD 405, 2017)	Six instead of three animals were used.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive (low purity: 46.1 % glyphosate), Category 3a **Conclusion AGG:** The study was conducted in accordance with OECD 405. However, it is noted that the purity is low and therefore this study is not considered relevant for the overall classification of glyphosate for eye irritation (supplementary).

In an eye irritation study, six New Zealand White rabbits were treated with glyphosate premix (Batch: 290-JaK-146-4, Purity: 46.1 % glyphosate). One animal was treated first; 0.1 mL of the undiluted test substance was instilled into the right conjunctival sac of the animal. Immediately before treatment, one drop of a local anaesthetic was instilled into both eyes of the animal to reduce the pain. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours. Based on the observations made in the first animal, a further five animals were treated following the same procedure.

Application of glyphosate premix into the rabbit eye resulted in effects on the iris and conjunctivae, which all resolved within 24-hours. The individual mean irritation scores (24 – 72-hours) of the rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate (with a purity of 46.1 %) is not irritating to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate premix (technical concentrate)
Description:	Pale yellow liquid
Lot/Batch #:	290-JaK-146-4
Purity:	62.2 % as glyphosate isopropylamine salt, 46.1 % as glyphosate
Stability of test compound:	Expiry date: 1995-09-30

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	12 – 20 weeks
Sex:	Male and Females
Body weight at dosing:	2.17 – 2.72 kg
Acclimation period:	At least 5 days
Diet/Food:	Stanrab SQC rabbit diet (Special Diets Services Ltd., Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in suspended metal cages
Environmental conditions:	Temperature: 18 – 21 °C Humidity: 44 – 57 % Air changes: 15 / hour Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1994-04-19 to 1994-04-23

Animal assignment and treatment:

Both eyes of all six animals (three males and three females) were examined for lesions with fluorescein sodium 24-hours before administration and again immediately before treatment with an ophthalmoscope.

One to two minutes prior to treatment with the test substance, one drop of local anaesthetic was instilled into both eyes of all animals, to minimise pain. A volume of 0.1 mL of glyphosate premix was placed into the conjunctival sac of the right eye of one animal. The eyelids were gently held together for one second, and then released to prevent loss of the article. The left eye served as control and remained untreated. Immediately after instillation of the test material, an assessment of pain was made. Based on the observed responses, five further animals were treated in the same way.

At 1, 24, 48 and 72-hours all eyes were examined for signs of irritation using a standard ophthalmoscope. Effects on cornea, iris, and conjunctivae (redness and chemosis) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea were observed in any animal. Effects on the iris were observed in 4/6 animals. Conjunctival redness and chemosis were observed in all animals. In addition, conjunctival discharge was observed in 5/6 animals. All effects on the eyes reversed within 24-hours.
A summary of the findings is given in the table below.

Table 6.2.5.14-8 Glyphosate premix: Acute eye irritation test in the rabbit. [redacted] 1994): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
57 (female)	1	0	0	1	1	1	0
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
12 (female)	1	0	0	0	1	1	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
55 (female)	1	0	0	1	1	1	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
59 (female)	1	0	0	1	1	1	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
65 (male)	1	0	0	1	2	1	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
101 (female)	1	0	0	0	1	1	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation that six instead of three animals were used, the study is in concordance with the current OECD TG 405 (2017). Therefore, the study is considered acceptable and the outcome can be reported as valid. Nevertheless, due to the low purity of the test substance (46.1 % as glyphosate), the reliability of the study is considered supplementary, only.
Instillation of Glyphosate premix into the rabbit eye elicited a slight irritation response after 1 hour, which was reversible within 24-hours. The individual mean scores at 24, 48 and 72-hours for the animals were 0.00 for corneal opacity, iris lesions, conjunctival redness and conjunctival chemosis.
Thus, under test conditions of the study, glyphosate is not an irritant to the eye.

Assessment and conclusion by RMS:
Due to the low purity of the test substance the study is considered supplementary.
The conclusion by the applicant is agreed.

B.6.2.5.15. Study 15

Data point:	CA 5.2.5/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Acute eye irritation test in the rabbit
Report No	710/18
Document No	Not reported
Guidelines followed in study	OECD 405 (1987)
Deviations from current test guideline (OECD 405, 2017)	A lower amount of test substance was used.
Previous evaluation	Not accepted in RAR (2015)
GLP	Uncertain
Acceptability/Reliability:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000 Category 4b **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered unacceptable due to the low amount of test substance used and the limited reporting.

Short description of study design and observations:	A single application of the test material to the non-irrigated eye of one rabbit was performed using 0.76 g of test substance (glyphosate technical, 95 % purity; batch not reported). A volume of 0.1 mL of the test material (approximately 76 mg) was placed into the right eye of one female albino rabbit (New Zealand White rabbit) by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for about one second after application, to prevent loss of the test material, and then released. The left eye remained untreated and was used for control purposes. Immediately after administration of the test substance, an assessment of the initial pain reaction was made. Assessment of ocular damage/irritation was made approximately 1 hour following treatment, according to the Draize scale. Any other ocular effects were also noted. Examination of the eye was facilitated by use of light source from a standard ophthalmoscope. Due to the severe eye reactions, test was stopped after 1 hour for humane reasons.
Short description of results:	Opaque corneal opacity, iridial inflammation and moderate conjunctival irritation were noted in the treated eye one hour after treatment. Other ocular effects noted were sloughing of the cornea, haemorrhage of the lower conjunctival membrane and blood stained discharge. The animal was killed for humane reasons immediately

	after the 1-hour observation in accordance with the UK Home Office guidelines. Scores at 1 hour post dosing: Cornea opacity: 4, iris: 1, conjunctival redness: 2, conjunctival chemosis: 2, discharge: 3 Further eye observations: Sloughing of the cornea, haemorrhage of the lower conjunctival membrane, blood stained discharge.

Assessment and conclusion by RMS:
The study is considered unacceptable due to the limited reporting and the low amount of test substance used. Therefore, no conclusion could be drawn.

B.6.2.5.16. Study 16

Data point:	CA 5.2.5/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate ([redacted]): Primary eye irritation study in rabbits
Report No	[redacted] 93-405/N
Document No	Not reported
Guidelines followed in study	OECD 405 (1981)
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during or after dosing to reduce the pain. Test item was applied to the centre of corneal surface instead of the conjunctivial sac. All animals were tested at the same time instead of using a tiered testing approach. Four animals were used in the test. Body weights, clinical signs, mortality, age, housing, and source of the animals were 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 but with restrictions (reliable with restrictions) due to the limited reporting.

In an eye irritation study, four female albino rabbits were treated with glyphosate (Batch: 36300892, Purity: 99.6 %) by instillation of 0.1 g of the undiluted test substance onto the centre of the cornea of one eye of each animal. The other untreated eye served as control. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48, 72, 96 hours and at 7 and 14 days.

Instillation of glyphosate into the rabbit eye resulted in effects on the cornea, iris and conjunctivae in all animals which reversed by Day 14. The individual mean irritation scores (24 – 72-hours) of the rabbits for the cornea, iris, and conjunctivae were as follows:

- for corneal opacity: 2.00, 1.00, 1.33, 1.00
- for iris lesions: 1.00, 1.00, 0.33, 1.00
- for conjunctival redness: 1.00, 1.67, 2.00, 2.00
- for chemosis of the conjunctiva: 2.00, 1.67, 2.00, 3.00

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate technical
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	99.6 %
Stability of test compound:	Expiry date: 1994-09-01

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	Not specified
Age:	Not specified
Sex:	Female
Initial body weight:	Not specified
Acclimation period:	5 days
Diet/Food:	Standard rabbit chow with fresh carrots, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Wire box (not further specified)
Environmental conditions:	Temperature: 18 ± 2 °C
	Humidity: 40 – 70 %
	Air changes: 10 / hour
	Photoperiod: 12-hour light / dark cycle

B: Study design and methods

In life dates: 1993-11-29 to 1993-12-12

Animal assignment and treatment:

24-hours prior to treatment, each eye of four female rabbits was examined with fluorescein-Na solution and a medical lamp. Only animals with intact conjunctivae, iris and cornea were used.

An amount of 0.1 g of the test substance was placed to the centre of the corneal surface of the right eye of each rabbit. The other eye served as control and remained untreated.

At 1, 24, 48, 72, 96 hours, 7 and 14 days post-administration, all eyes were examined for signs of irritation. Effects on cornea, iris, and conjunctivae (redness, chemosis, and discharge) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

After 1 hour, effects on the cornea, iris, and conjunctivae were observed in all four animals. Effects on the iris were resolved by Day 7 while effects on the cornea and conjunctivae were resolved by Day 14.

A summary of the findings is given in the table below.

Table 6.2.5.16-9 Primary eye irritation study in rabbits [redacted] 1994): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (female)	1	2	4	1	1	2	1
	24	**2**	4	**1**	**1**	**2**	3
	48	**2**	4	**1**	**1**	**2**	3
	72	**2**	4	**1**	**1**	**2**	3
	Day 4	2	4	1	1	2	1
	Day 7	1	1	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**2.00**	-	**1.00**	**1.00**	**2.00**	-
Rabbit 2 (female)	1	1	4	1	2	2	1
	24	**1**	4	**1**	**2**	**2**	1
	48	**1**	4	**1**	**2**	**2**	1
	72	**1**	4	**1**	**1**	**1**	0
	Day 4	1	4	0	1	1	1
	Day 7	0	0	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	-	**1.00**	**1.67**	**1.67**	-
Rabbit 3 (female)	1	2	4	1	2	2	2
	24	**2**	4	**1**	**2**	**2**	2
	48	**1**	4	**0**	**2**	**2**	2
	72	**1**	4	**0**	**2**	**2**	2
	Day 4	1	4	0	1	1	1
	Day 7	0	0	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.33**	-	**0.33**	**2.00**	**2.00**	-
Rabbit 4 (female)	1	2	4	1	2	3	2
	24	**1**	4	**1**	**2**	**3**	3
	48	**1**	4	**1**	**2**	**3**	3
	72	**1**	4	**1**	**2**	**3**	3
	Day 4	1	4	0	1	1	1
	Day 7	1	2	0	1	1	1
	Day 14	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	-	**1.00**	**2.00**	**3.00**	-

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study is in concordance with the current OECD TG 405 (2017) except for minor deviations that are not expected to influence the study outcome such as: No anaesthetic was given prior, during or after dosing to reduce the pain and all animals were tested at the same time instead of using a tiered testing approach.

The test item was applied directly to the centre of corneal surface instead of the conjunctival sac. However, this deviation is regarded as a worst-case scenario. The study is therefore considered valid and acceptable. The individual mean scores at 24, 48 and 72-hours for the animals were 2.00, 1.00, 1.33, 1.00 for corneal opacity,

1.00, 1.00, 0.33, 1.00 for iris lesions, 1.00, 1.67, 2.00, 2.00 for conjunctival redness, and 2.00, 1.67, 2.00, 3.00 for conjunctival chemosis.
Thus, under test conditions of the study, glyphosate has the potential to cause serious eye damage.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the study conditions the test substance is irritating to the rabbit eyes.

B.6.2.5.17. Study 17

Data point:	CA 5.2.5/016
Report author	[redacted]
Report year	1991
Report title	Primary eye irritation study with glyphosate technical (FSG 03090 H/05 March 90) in New Zealand White rabbits
Report No	[redacted] 79.EYE
Document No	Not reported
Guidelines followed in study	OECD 405 (1987), US EPA 81-4 (1984)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during, or after test substance application. Individual scores for eye irritation not included in the report, therefore no mean values could be obtained. A tier testing approach was not used.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Supportive, Category 3a **Conclusion AGG:** The study is considered unacceptable due to the limitations

In an eye irritation study, 0.1 g of the undiluted test substance (glyphosate technical, Batch: 60, Purity: 96.8 %) was instilled into the right conjunctival sac of three New Zealand White rabbits (two males and one female). The eyes were rinsed after 24-hours with distilled water to remove the test substance. Animals were observed for 21 days for mortality and signs of eye irritation. Eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours and 7, 14 and 21 days after test item instillation.

The female rabbit was found dead on Day 2 post-exposure. Application of glyphosate technical into the rabbit eye resulted in corneal opacity (grade 2) in all animals persisting until death or the end of the study. Furthermore, one male rabbit showed ulceration from Day 14 until end of the study. Redness (grade 1 or 2) of the eye was observed in two animals until Day 7, thereafter one animal recovered while redness of the eye persisted in the other animal until termination of the study. Chemosis (grade 1 or 2) was observed in all animals until death or end of the study. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 2.00, 2.00 (score for the third animal not available due to its death after the 48 hour scoring)
- for iris lesions: 0.00, 0.00 (score for the third animal not available due to its death after the 48 hour scoring)
- for conjunctival redness: 1.44 (mean for all three animals due to missing information on individual scores)
- for chemosis of the conjunctiva: 1.11 (mean for all three animals due to missing information on individual scores)

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate Technical
Description:	Solid white coloured crystals, odourless
Lot/Batch #:	60
Purity:	96.8 %
Stability of test compound:	More than two years; expiry date 1992-07

2. **Vehicle and/ or positive control:** None

3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Approximately 14 weeks
Sex:	Male and Female
Weight at dosing:	1.7 – 2.1 kg
Acclimation period:	At least 4 days
Diet/Food:	Standard "Gold Mohur" brand pelleted rabbit maintenance diet (M/S Lipton India Ltd., Bangalore, India), *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV light, *ad libitum*
Housing:	Individually in stainless steel / aluminium cages equipped for pelleted feed and drinking water
Environmental conditions:	Temperature: 23 ± 2 °C
	Humidity: 68 ± 6 %
	Air changes: 10 – 15 / hour
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1990-09 to 1990-10 (specific dates not reported)

Animal assignment and treatment:
Two male and one female rabbit were used for this test. Both eyes of each animal were examined before administration.

An amount of 0.1 g of finely ground glyphosate technical was placed into the conjunctival sac of the left eye. The eyelids were gently held together for one second and then released to prevent loss of the test article. The right eye served as control and remained untreated. After 24-hours of exposure, the eye was rinsed with distilled water to remove the test substance.

At 1, 24, 48 and 72-hours and at days 7, 14 and 21 after instillation of the test substance, all eyes were inspected for irritation according to the Draize criteria. Clinical signs on cornea, iris and conjunctivae, as well as chemosis were recorded and investigated for their reversibility. Furthermore, the animals were checked for mortality every day and individual body weights were recorded on the first and last day of the study. A gross pathological examination was performed at death or at study termination.

Results

A. MORTALITY

The female rabbit died on Day 2 after treatment.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT

Decrease in body weight was observed for all three animals.

D. NECROPSY

Necropsy findings showed opacity of the treated eye in all animals, involving 3/4 or 1/2 of the area, and one male rabbit showed ulceration. Enteritis was seen in the deceased animal (female).

E. EYE OBSERVATIONS

All animals showed signs of opacity (grade 2) from 24-hours after instillation, which persisted either until death or until study termination (Day 21). Furthermore, one male rabbit showed ulceration beginning from Day 14 until end of the study. None of the animals showed iridial reactions. Redness (grade 1 or 2) of the eye persisted until Day 7 in two animals. One animal recovered while redness persisted in the other animal until end of the study in the other animal. Chemosis (grade 1 or 2) was seen in all animals from the beginning until death or end of the study.

A summary of the findings is given in the table below.

Table 6.2.5.17-10 Primary eye irritation study with glyphosate technical (FSG 03090 H/05 March 90) in New Zealand White rabbits [redacted] 1991): Eye irritation – Individual irritation scores

Effects	Score	1 h	24 h	48 h	72 h [a]	Mean (24h, 48h, 72h)	Day 7	Day 14	Day 21
Cornea	0	-	-	-	-	Animal 1: 2.00 Animal 2: 2.00 Animal 3: NA [a]	-	-	-
	1	-	-	-	-		-	-	-
	2	3/3	3/3	3/3	2/2		2/2	2/2	2/2
	3	-	-	-	-		-	-	-
	4	-	-	-	-		-	-	-
Iris	0	3/3	3/3	3/3	2/2	Animal 1: 0.00 Animal 2: 0.00 Animal 3: NA [a]	2/2	2/2	2/2
	1	-	-	-	-		-	-	-
	2	-	-	-	-		-	-	-
Conjunctiva Redness	0	-	-	-	-	Mean of all two / three animals: 1.44 [a,b]	1/2	1/2	1/2
	1	1/3	1/3	1/3	1/2		1/2	1/2	1/2
	2	2/3	2/3	2/3	1/2		-	-	-
	3	-	-	-	-		-	-	-
Conjunctiva Chemosis	0	-	-	-	-	Mean of all two / three animals: 1.11 [a,b]	-	-	-
	1	2/3	1/3	3/3	2/2		2/2	2/2	2/2
	2	1/3	2/3	-	-		-	-	-
	3	-	-	-	-		-	-	-

	4	-	-	-	-		-	-	-

[a] One animal (female) died on Day 2 post application.
[b] Individual calculation not possible as no individual animal data were provided.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, no stepwise approach and the missing possibility for calculation individual mean scores, the study is in concordance with the current OECD TG 405 (2017). The study is considered acceptable and the outcome can be reported as valid. Nevertheless, due to the missing calculation of the individual mean scores, the reliability of the study is considered supplementary, only.
Instillation of glyphosate into the rabbit eye elicited a response in the eye of the treated animals. No individual mean scores at 24, 48 and 72-hours could be calculated. However, due to the lack of reversibility, glyphosate has the potential to causes serious eye damage under the test conditions of the study.
Based on the effects on cornea and conjunctiva, which were not reversible within 21 days, the test substance requires classification as "Serious Eye Damage, Category 1 (H318)" according to the classification criteria laid down in the CLP Regulation (EC No. 1272/2008).

Assessment and conclusion by RMS:
The study is considered unacceptable due to the limited reporting and the missing individual scores for eye irritation. Therefore, no conclusion could be drawn. However, considering that eye irritation was observed up to day 21 of the study it seems classification for Eye Damage (H318) would be appropriate on the basis of the study.

B.6.2.5.18. Study 18

Data point:	CA 5.2.5/017
Report author	[redacted]
Report year	1991
Report title	Acute eye irritation study in New Zealand White rabbits treated with the test article glyphosate technico 98 %
Report No	910260
Document No	PRO496
Guidelines followed in study	EEC guidelines
Deviations from current test guideline (OECD 405, 2017)	Number of animals (6), no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Study report is limited and the batch of the test substance is not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP	No
Acceptability/Reliability:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000 Category 4b **Conclusion AGG:** In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study is considered unacceptable since the study was not performed according to GLP

Short description of study design and observations:	The eyes of three male (New Zealand White) rabbits were treated with a single dose of 100 mg test substance (glyphosate acid, purity 98.0 %; batch not reported) into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about 3-4 seconds in order to prevent loss of the material. The other eye, remained untreated and served as control. The eyes of the test animals were washed out at 24 hours following the test article installation. Observations of the eyes were made at 1, 24, 48 and 72 hours after application. After the 24

	hour reading the cornea were not examined after instillation of one drop 1% sodium fluorescein due to the severe chemosis of the lids. The ocular reaction was scored according to the Draize scale.
Short description of results:	No animals died during the observation period and no clinical signs or behavioral alterations were seen in any animals. Locally the test compound induced moderate lacrimation 24 hours after treatment and whitish material in the inferior conjunctival fornix at the 48 and 72 hour observation in all treated rabbits. Several ocular effects (score 1 and 2) in all animals which were reversible within 3 days. The results are reported below.

Table 6.2.5.18-11 Individual eye irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
1 (male)	1	0	n.r.	0	2	1	n.r.
	24	**2**	n.r.	**1**	**2**	**2**	n.r.
	48	**1**	n.r.	**1**	**2**	**1**	n.r.
	72	**0**	n.r.	**0**	**1**	**0**	n.r.
	7 days	0	n.r.	0	0	0	n.r.
	14 days	0	n.r.	0	0	0	n.r.
	21 days	0	n.r.	0	0	0	n.r.
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.67**	**1.67**	**1.00**	-
2 (male)	1	0	n.r.	0	2	1	n.r.
	24	**2**	n.r.	**1**	**2**	**2**	n.r.
	48	**1**	n.r.	**1**	**2**	**1**	n.r.
	72	**0**	n.r.	**0**	**1**	**0**	n.r.
	7 days	0	n.r.	0	0	0	n.r.
	14 days	0	n.r.	0	0	0	n.r.
	21 days	0	n.r.	0	0	0	n.r.
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.67**	**1.67**	**1.00**	-
3 (male)	1	0	n.r.	0	2	1	n.r.
	24	**2**	n.r.	**1**	**2**	**2**	n.r.
	48	**1**	n.r.	**1**	**2**	**1**	n.r.
	72	**0**	n.r.	**0**	**1**	**0**	n.r.
	7 days	0	n.r.	0	0	0	n.r.
	14 days	0	n.r.	0	0	0	n.r.
	21 days	0	n.r.	0	0	0	n.r.
Individual mean (24, 48, 72 h)		**1.00**	**-**	**0.67**	**1.67**	**1.00**	-

Nr: not reported.

Assessment and conclusion by RMS:
The study is considered unacceptable since it was not performed according to GLP. Therefore, no conclusion could be drawn.

B.6.2.5.19. Study 19

Data point:	CA 5.2.5/019
Report author	[redacted]
Report year	1990
Report title	Acute eye irritation/corrosion of glyphosate technical in the rabbit

Report No	[redacted]900822
Document No	[redacted]002
Guidelines followed in study	OECD 405 (1981), B5 Annex to EEC Directive 84/449/EEC
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. New conclusion drawn based on the current CLP regulation (EC No. 1272/2008). Environmental conditions, acclimation period, and age of animals were not specified. No tiered testing approach was used; instead all three animals were tested together. Individual data on clinical signs were not reported. Bodyweight data was not investigated.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting

In an eye irritation study, 0.1 g of the undiluted test substance (glyphosate technical, Batch: 0190 A, Purity: 98.1 %) was instilled into one conjunctival sac of three New Zealand White rabbits (3 females). Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours and daily thereafter until Day 8 of the study period.

Application of glyphosate technical into the rabbit eye resulted in corneal opacity in all animals persisting until Day 7. Furthermore, one rabbit showed effects on the iris until Day 6. Conjunctivae redness was observed in all animals until Day 6, while chemosis reversed 72-hours after treatment. The individual mean irritation scores (24 – 72-hours) of the three rabbits were as follows:

- for corneal opacity: 1.00, 1.00, 1.67
- for iris lesions: 0.00, 0.00, 0.67
- for conjunctival redness: 1.00, 1.00, 1.33
- for chemosis of the conjunctiva: 0.67, 0.67, 1.00

Based on the study, glyphosate technical is irritating to the rabbit eye under the chosen test conditions.

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:	Provided by the sponsor

2. **Vehicle and/ or positive control:** None
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]

Age:	Adult (not specified)
Sex:	Females
Weight at dosing:	2.1 – 3.2 kg
Acclimation period:	Not specified
Diet/Food:	Standard rabbit diet (Redmills, Goresbridge, Co. Kilkenny, Ireland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in standard rabbit cages without bedding material. Grid bottom cages used.
Environmental conditions:	Temperature: Not recorded
	Humidity: Not recorded
	Air changes: Not specified
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1990-03-30 to 1990-08-22

Animal assignment and treatment:
Three female rabbits were used for this test. Both eyes of each animal were examined before administration and only animals showing no signs of eye irritation, ocular defects or pre-existing corneal injury were used for the study.

An amount of 0.1 g of glyphosate technical was placed into the conjunctival sac of one eye. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. The animals were restrained during the instillation of the test substance and for a further hour after instillation.

At 1, 24, 48 and 72-hours and daily thereafter until Day 8 post-instillation of the test substance, all eyes were inspected for irritation according to the Draize criteria. Clinical signs on cornea, iris and conjunctivae, as well as chemosis were recorded and investigated for their reversibility. Furthermore, the animals were observed for lesions and toxic effects.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

All animals showed signs of opacity (up to grade 2) from 1 hour after instillation, which had resolved until Day 8 of the study. One rabbit showed a reaction on the iris beginning 48 hours after treatment and persisting until Day 7. All three rabbits showed some degree of conjunctival redness, which cleared bay Day 7. Chemosis was observed in all animals beginning 1 hour after treatment and returning to normal after 48 hours. All signs of irritation were clear by Day 8.

A summary of the findings is given in the table below.

Table 6.2.5.12-1 Acute eye irritation/corrosion of glyphosate technical in the rabbit [redacted] 1990): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva	
				Redness	Chemosis
BL-623 (female)	1	1	0	0	3
	24	**1**	**0**	**1**	**1**
	48	**1**	**0**	**1**	**1**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	1	0
	Day 6	0	0	1	0
	Day 7	0	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**0.00**	**1.00**	**0.67**
BL-628 (female)	1	1	0	0	3
	24	**1**	**0**	**1**	**1**
	48	**1**	**0**	**1**	**1**
	72	**1**	**0**	**1**	**0**
	Day 4	1	0	1	0
	Day 5	1	0	1	0
	Day 6	1	0	1	0
	Day 7	1	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.00**	**0.00**	**1.00**	**0.67**
BL-767 (female)	1	1	0	0	3
	24	**1**	**0**	**1**	**2**
	48	**2**	**1**	**1**	**1**
	72	**2**	**1**	**2**	**0**
	Day 4	2	2	2	0
	Day 5	2	2	2	0
	Day 6	1	1	1	0
	Day 7	1	0	0	0
	Day 8	0	0	0	0
Individual mean (24, 48, 72 h)		**1.67**	**0.67**	**1.33**	**1.00**

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, no rinsing 1 hour after application and no reporting of environmental conditions, the study is in concordance with the current OECD TG 405 (2017). As the lack of rinsing is considered as worst-case, this deviation is considered to compromise the outcome of the study. Nevertheless, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited slight to moderate response in treated animals. The individual mean scores at 24, 48 and 72-hours for the animal were 1.00, 1.00, 1.67 for corneal opacity, 0.00, 0.00, 0.67 for iris lesions, 1.00, 1.00, 1.33 for conjunctival redness, and 0.67, 0.67, 1.00 for conjunctival chemosis.

Thus, under test conditions of the study, glyphosate is considered as irritating to the eyes.

Assessment and conclusion by RMS:

The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the study conditions the test substance is irritating to the rabbit eyes.

B.6.2.5.20. Study 20

Data point:	CA 5.2.5/020
Report author	[redacted]
Report year	1989
Report title	Glyphosate technical: Primary eye irritation test in rabbits
Report No	5886
Document No	243268
Guidelines followed in study	US EPA 81-4
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Observation period terminated after 4 days instead of 21 days. Age and weight of animals and number of air changes per hour not specified. Only one animal was tested. Data on clinical signs and bodyweight is not reported. The stability of the test chemical is not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) since only one animal is tested.

In an eye irritation study, 0.1 g of the undiluted test substance (glyphosate technical, Batch: 206-Jak-25-1, Purity: 98.6 %) was instilled into the right conjunctival sac of one New Zealand White rabbit. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48, 72-hours and 4 days. The study was terminated after 4 days, and due to the degree of eye irritation observed in this initial animal, no further animals were treated.

Application of glyphosate technical into the rabbit eye resulted in corneal opacity, effects on the iris and conjunctivae which all resolved after 72-hours, except for corneal opacity which persisted until the end of the study period. The individual mean irritation scores (24 – 72-hours) of the rabbit were as follows:

- for corneal opacity: 1.00
- for iris lesions: 1.00
- for conjunctival redness: 2.00
- for chemosis of the conjunctiva: 2.00

Based on the study, glyphosate has the potential to causes serious eye damage to the eye under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate Technical (PMG)
Description:	White powder
Lot/Batch #:	206-Jak-25-1
Purity:	98.6 %
Stability of test compound:	Not specified

2. **Vehicle and/ or positive control:** None
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Males and females (nulliparous and non-pregnant)
Weight at dosing:	Not specified
Acclimation period:	6 days
Diet/Food:	Standard rabbit diet (Special Diets Services, Witham, Essex, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in aluminium cages with grid floors beneath which were peat moss filled trays
Environmental conditions:	Temperature: 17 – 19 °C
	Humidity: 61 % (mean)
	Air changes: Not specified
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1989-06-26 to 1988-07-01

Animal assignment and treatment:

Three male and three female rabbits were prepared for the study. Both eyes of all animals were examined 24-hours before administration. One rabbit was treated first.

An amount of 0.1 g of glyphosate technical was placed into the right conjunctival sac of one eye. The eyelids were gently held together for two seconds and then released to prevent loss of the article. The left eye served as control and remained untreated.

At 1, 24, 48, 72-hours and 4 days, the rabbit's eyes were examined for signs of irritation, using a Panoramic Loupe and pen torch. Effects on cornea, iris and conjunctivae (redness and chemosis) were scored using the Draize criteria. Due to severe irritating effects, the remaining five animals were not treated.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

The rabbit showed signs corneal opacity, covering half the eye, up to 48 hours after treatment. Effects on the iris were observed between 24-hours and 72-hours. In addition, conjunctival redness and chemosis were observed between 1 hour and 72-hours after treatment. A slight to moderate discharge was seen up to 72-hours. The

observations resolved after 72-hours, except for corneal opacity which persisted until termination of the study (Day 4). Because of the degree of response, no further animals were treated.

A summary of the findings is given in the table below.

Table 6.2.5.13-1 Glyphosate technical: Primary eye irritation test in rabbits. [redacted] 1989): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
1 (male)	1	D [a]	0	0	2	2	1
	24	**D** [a]	0	**1**	**2**	**3**	2
	48	**1**	2	**1**	**2**	**2**	1
	72	**1**	2	**1**	**2**	**1**	1
	Day 4	**1**	2	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**1.00** [b]	**-**	**1.00**	**2.00**	**2.00**	**-**

[a] Dullness of cornea over whole area
[b] Mean of 48 and 72 as no value was given for 24 h

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). This deviation is considered not to compromise the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate into the rabbit eye elicited a strong response in the treated animal. The individual mean scores at 24 and 72-hours for the animals were 1.00 for corneal opacity and iris lesions, and 2.00 for conjunctival redness and conjunctival chemosis.
Thus, under test conditions of the study, glyphosate is considered to causes serious eye damage.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions), since only one animal was tested. According to the current rules for classification the test substance would be considered to cause serious eye damage under the study conditions since the effects were not fully reversible by the end of the study period.

B.6.2.5.21. Study 21

Data point:	CA 5.2.5/021
Report author	[redacted]
Report year	1989
Report title	Primary eye irritation with glyphosate technical (isopropylamine salt 62 % in water equivalent to 46 % of N-phosphonomethylglycine acid) in the rabbit (rinsed / unrinsed)
Report No	238083
Document No	PRO423
Guidelines followed in study	OECD 405 (1987), EPA (1984)
Deviations from current test guideline (OECD 405, 2017)	Low purity, Number of animals (6), no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Irrigation after 30 seconds.
Previous evaluation	Not accepted in RAR (2015)
GLP	Yes
Acceptability/Reliability:	**Conclusion GRG:** Full study report is not available to the applicant. Only a short summary is provided in the Monograph, B5, 2000, *Category 4b* **Conclusion AGG:** In contrast to the applicant, the AGG has access to

	the study report and the summary written above was prepared by the AGG. The study is considered unacceptable due to the limited reporting.

Short description of study design and observations:	The eyes of three males and three females were treated with a single dose of 0.1 mL test substance (glyphosate technical, purity 62 % in water equivalent to 46% of N-phosphonomethylglycine acid, batch number 21/39) into the conjunctival sac of the left eye of the rabbit and the lid was gently closed for a few seconds. The eyes of the females were rinsed with lukewarm physiological saline approximately 30 seconds after treatment while the eyes of the males remained unrinsed. The eyes of each animal were examined 1, 24, 48 and 72 hours after administration. The irritation was assessed according to the Draize scoring system. Mortality was checked daily and bodyweights were recorded at the start of the acclimatization, day 1 of test (application day) and at termination of observation. The observation was terminated 72 hours after administration of the test article. All rabbits were killed by an intravenous injection of T61 into the ear vein and discarded.
Short description of results:	Redness of the conjunctivae observed 24-hours after treatment. Only the results of the unrinsed eyes are reported below.

Table 6.2.5.21-14 Individual eye irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
20 (male)	1	0	n.r.	0	1	1	n.r.
	24	**0**	n.r.	**0**	**1**	**0**	n.r.
	48	**0**	n.r.	**0**	**0**	**0**	n.r.
	72	**0**	n.r.	**0**	**0**	**0**	n.r.
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
21 (male)	1	0	n.r.	0	1	2	n.r.
	24	**0**	n.r.	**0**	**1**	**0**	n.r.
	48	**0**	n.r.	**0**	**0**	**0**	n.r.
	72	**0**	n.r.	**0**	**0**	**0**	n.r.
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
22 (male)	1	0	n.r.	0	1	1	n.r.
	24	**0**	n.r.	**0**	**0**	**0**	n.r.
	48	**0**	n.r.	**0**	**0**	**0**	n.r.
	72	**0**	n.r.	**0**	**0**	**0**	n.r.
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-

Nr: not reported.

Assessment and conclusion by RMS:
Due to the limited reporting and the wash step after 30 seconds the study is considered unacceptable. Therefore, no conclusion could be drawn.

B.6.2.5.22. Study 22

Data point:	CA 5.2.5/022
Report author	[redacted]
Report year	1988

Report title	Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits
Report No	88.2053.009
Document No	[redacted]88-29
Guidelines followed in study	US EPA 81-4 (1984)
Deviations from current test guideline (OECD 405, 2017)	No treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application. Six animals were dosed at once instead of three animals in a step wise approach as required by the current OECD guideline 405 (2017). Irrigation took place after 24-hours instead of after one hour. Clinical signs, bodyweight and necropsy findings were not reported. The age of the animals was not reported. The number of air changes was not reported and the temperature was slightly outside the specified range.
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) due to the limited reporting.

In an eye irritation study, 0.1 g of the undiluted glyphosate (Batch: XLI-55, Purity: 97.76 %) was instilled into the conjunctival sac of one eye of six young adult New Zealand rabbits whereas the second eye served as control. Animals were observed for 21 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72-hours and 7, 14 and 21 days after test substance instillation.

Under the conditions of this study, glyphosate produced corneal opacity and conjunctival irritation with blistering in all rabbits after test material instillation. Three rabbits exhibited pannus on the cornea, one rabbit had prominent vascularization of the conjunctiva, and another animal had a blood-like discharge. One rabbit was found dead 20 days after dose administration. However, this death was not considered treatment related. Corneal opacity persisted through study day 21 (termination) in three of five (3/5) animals. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration. The individual mean irritation scores (24 – 72-hours) of the six rabbits were as follows:

- for corneal opacity: 2.67, 1.67, 2.00, 1.00, 2.33, 2.67
- for iris lesions: 0.00, 0.00, 1.00, 0.00, 0.00, 0.00
- for conjunctival redness: 2.00, 2.00, 2.00, 2.00, 2.00, 2.00
- for conjunctival chemosis: 2.00, 3.33, 3.33, 2.67, 2.00, 2.00

Based on the study, glyphosate TC was seriously damaging to the eyes under the chosen test conditions.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76 %
Stability of test compound:	Stored at room temperature

2. **Vehicle and/ or positive control:** None
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult
Sex:	Males and females
Weight at dosing:	Between 2 – 3 kg
Acclimation period:	At least five days
Diet/Food:	NIH 09 Rabbit Ration certified feed (Zeigler Brothers, Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9 ºC
	Humidity: 40 – 60 %
	Air changes: Not specified
	Photocycle: 12 hours light / dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1988-04-11 to 1988-05-02

Animal assignment and treatment:

The test was conducted using six young adult New Zealand albino rabbits. The test substance (0.1 g) was instilled into one eye of each rabbit. The lower eyelid was pulled gently away from the eyeball to form a cup (conjunctival sac) and the test substance inserted therein. The lids were then held together for one second and released. Following scoring at 24-hours after dose administration, any residual material was rinsed from the eye with physiological saline. Treated and untreated eyes were examined at 1, 24, 48 and 72-hours and 7, 14 and 21 days after test substance instillation. The cornea, iris, and conjunctiva were scored separately according to the Draize system. The animals were observed twice daily for mortality at least five hours apart. Body weights were determined on study day 1 prior to dose administration and at death. At study termination, surviving animals were sacrificed.

Results

A. MORTALITY

One rabbit was found dead 20 days after dose administration. Prior to death, this animal exhibited anorexia, and gross necropsy revealed a clear gel-like substance in the large intestine. These findings are consistent with mucoid enteropathy, a condition occasionally noted in stock laboratory rabbits. Therefore, the death was considered spontaneous and unrelated to treatment.

B. CLINICAL OBSERVATIONS

Not reported.

C. BODY WEIGHT

Not reported.

D. NECROPSY

Not reported.

E. EYE OBSERVATIONS

At one hour after test substance instillation, all animals exhibited conjunctival irritation (redness, swelling, blistering and discharge). Corneal opacity was noted one hour after test substance instillation in four of six animals. Corneal opacity and conjunctival irritation were noted in all rabbits at the 24, 48 and 72-hour and 7 day examinations. Three rabbits exhibited pannus on the cornea; two eyes (iris) had sluggish reactions to light; one rabbit had prominent vascularisation of the conjunctival, and another animal had a blood-like discharge. Corneal opacity persisted through study termination (Day 21) in three of five rabbits. Of the remaining two rabbits, one exhibited slight conjunctival discharge at study termination and the other rabbit's treated eye appeared normal 14 days after dose administration.

The individual mean irritation scores (24 – 72-hours) were calculated to be 2.67, 1.67, 2.00, 1.00, 2.33 and 2.67 for corneal opacity, 0.00, 0.00, 1.00, 0.00, 0.00, 0.00 for iris lesions, 2.00 for all animals for conjunctival redness, and 2.00, 3.33, 3.33, 2.67, 2.00, 2.00 for conjunctival chemosis. The individual scores for each time point, individual mean and group mean scores (24 – 72-hours) are presented in the table below.

Table 6.2.5.22-15 Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits [redacted]., 1988c): Eye irritation – Individual irritation scores

Animal No.	Scoring[a] [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1 (88 – 1181)	1	2	1	0	2	2 [b]	2
	24	**2**	3	**0**	**2**	**2 [b]**	2
	48	**3**	1	**0**	**2**	**2 [b]**	2 [c]
	72	**3 [d]**	1	**0**	**2**	**2 [b]**	2
	7 days	3 [d]	1	0	2	1	0
	14 days	2	1	0	1	0	0
	21 days	2	1	0	0	0	0
Individual mean 24-72 h		**2.67**	**---**	**0.00**	**2.00**	**2.00**	**---**
Rabbit 2 (88 – 1182)	1	2	1	0	2	2 [b]	3
	24	**2**	2	**0**	**2**	**4 [b]**	3
	48	**2**	2	**0**	**2**	**4 [b]**	2
	72	**1**	1	**0**	**2**	**2 [b]**	1
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	1
	21 days	0	0	0	0	0	1
Individual mean 24-72 h		**1.67**	**---**	**0.00**	**2.00**	**3.33**	**---**
Rabbit 3 (88 – 1183)	1	2	1	0	2	2 [b,e]	2
	24	**2**	2	**1**	**2**	**4 [b,e]**	2
	48	**2**	1	**1**	**2**	**3 [b,e]**	2
	72	**2**	1	**1**	**2**	**3 [b,e]**	2
	7 days	3 [d]	2	1	3	2 [b]	2
	14 days	2	1	1	1	0	0
	21 days	2	1	0	0	0	0
Individual mean 24-72 h		**2.00**	**---**	**1.00**	**2.00**	**3.33**	**---**
Rabbit 4 (88 – 1258)	1	0	0	0	2	3 [b]	3
	24	**1**	4	**0**	**2**	**4 [b]**	3
	48	**1**	3	**0**	**2**	**2 [b]**	2
	72	**1**	3	**0**	**2**	**2 [b]**	2
	7 days	3	1	0	2	2	1
	14 days	0	0	0	0	0	0

Table 6.2.5.22-15 Primary Eye Irritation Study of Glyphosate Batch/Lot/NBR No. XLI-55 in New Zealand White Rabbits ([redacted] 1988c): Eye irritation – Individual irritation scores

Animal No.	Scoring[a] [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
	21 days	0	0	0	0	0	0
Individual mean 24-72 h		**1.00**	**---**	**0.00**	**2.00**	**2.67**	**---**
Rabbit 5 (88 – 1260)	1	0	0	0	2	2 [b]	2
	24	**2**	2	**0**	**2**	**2 [b]**	2
	48	**2**	2	**0**	**2**	**2 [b]**	2
	72	**3**	1	**0**	**2**	**2 [b]**	1
	7 days	4 [d]	1	1	2	2	1
	14 days	3 [d]	1	1	2	1	0
	21 days	3 [d]	1	0	1	0	0
Individual mean 24-72 h		**2.33**	**---**	**0.00**	**2.00**	**2.00**	**---**
Rabbit 6 (88 – 1261)	1	2	1	0	2	2 [b]	3
	24	**2**	2	**0**	**2**	**2 [b]**	2
	48	**3**	1	**0**	**2**	**2 [b]**	2
	72	**3**	1	**0**	**2**	**2 [b]**	0
	7 days	2	1	0	1	1	0
	14 days	2	1	0	0	0	0
	21 days	- [f]	-	-	-	-	-
Individual mean 24-72 h		**2.67**	**---**	**0.00**	**2.00**	**2.00**	**---**
Group mean 24 – 72 h		**2.06**	**---**	**0.17**	**2.00**	**2.56**	**---**

[a] Scores for treated eyes; untreated eyes appeared normal at all times.
[b] Mucus membrane of the eyelid appeared blistered.
[c] Blood-like discharge noted.
[d] Pannus on the cornea.
[e] Prominent vascularisation of the conjunctiva.
[f] Animal found dead 20 days after dose administration.

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and some minor deviations the study is in concordance with the current OECD TG 405 (2017). These deviations did not compromise the acceptability of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.
Instillation of glyphosate into the rabbit eye elicited strong response in treated animals. The individual mean scores over 24, 48 and 72-hours for each animal were calculated to be 2.67, 1.67, 2.00, 1.00, 2.33 and 2.67 for corneal opacity, 0.00, 0.00, 1.00, 0.00, 0.00, 0.00 for iris lesions, 2.0 for all animals for conjunctival redness and 2.00, 3.33, 3.33, 2.67, 2.00, 2.00 for conjunctival chemosis. Some effects were not reversible within 21 days.
Thus, glyphosate is considered to have the potential to seriously damage the eye.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the study conditions the test substance is damaging to the rabbit eye.

B.6.2.5.23. Study 23

Data point:	CA 5.2.5/023
Report author	[redacted]
Report year	1987
Report title	Primary eye irritation study of MON 8722 in New Zealand White rabbits
Report No	9307A
Document No	Not reported

Guidelines followed in study	US EPA 81-4 (1982)
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Sex nor the age of the animals was not specified. The stability of the test chemical is not reported. The number of air changes was not specified. Mean values had to be recalculated based on the current CLP regulation (EC No. 1272/2008). The purity of the test substance is low.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered supplementary due to the low purity making the study less relevant for the classification of glyphosate as approved in the EU.

In an eye irritation study, six New Zealand White rabbits were treated with glyphosate sodium salt (MON 8722) by instillation of 0.1 g of the undiluted test substance into the conjunctival sac of one eye of each animal. The eyes were rinsed 24-hours after treatment with physiological saline. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours. Furthermore, the animals were observed for mortality and systemic toxicity daily. At the end of the study period, all animals were killed by intracardiac injection of sodium pentorbarbital.

Instillation of MON 8722 into the rabbit eye resulted in effects on the conjunctivae in all animals, which resolved within 48 hours. The individual mean irritation scores (24 – 72-hours) of the rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.33, 0.00, 0.00, 0.00, 0.00, 0.00
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate sodium salt is not irritating to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate sodium salt (MON 8722)
Description:	White powder
Lot/Batch #:	XLG-256
Purity:	70.7 %
Stability of test compound:	Not specified

2. **Vehicle and/or positive control:** None
3. **Test animals:**

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)

Sex:	Not specified	
Initial body weight:	1.8 – 2.3 kg	
Acclimation period:	At least 5 days	
Diet/Food:	NIH 09 rabbit ration, certified feed, Zeigler Brothers, Inc., Gardners, PA, *ad libitum*	
Water:	Tap water, *ad libitum*	
Housing:	Individually in wire-mesh cages	
Environmental conditions:	Temperature:	20 – 23 °C
	Humidity:	40 – 60 %
	Air changes:	Not specified
	Photoperiod:	12 hours light / dark cycle

B: Study design and methods

In life dates: 1986-11-05 to 1986-11-08

Animal assignment and treatment:

Both eyes of all six animals were examined for lesions with fluorescein sodium and an ultraviolet lamp 24-hours before administration.

An amount of 0.1 g of the test substance was placed into the conjunctival sac of one eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. Twenty-four hours after the treatment and after scoring, the eyes were rinsed with physiological saline to remove any remaining test material.

At 1, 24, 48 and 72-hours all eyes were examined for signs of irritation. Effects on cornea, iris, and conjunctivae (redness, chemosis, and discharge) were scored using the Draize criteria. In addition, all animals were observed for mortality and systemic toxicity, daily. The body weights were measured immediately prior to dosing on study day 1. All animals were euthanized by intracardiac injection of sodium pentobarbital at the end of the study period.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea an on the iris were observed in any animal. Conjunctival redness, chemosis, and discharge were seen in all animals after 1 hour, however these observations resolved within 48 hours. In addition, blistering of the mucus membrane appeared blistered at 1 hour only. All effects were reversible.

A summary of the findings is given in the table below.

Table 6.2.5.23-16 Primary eye irritation study of MON 8722 in New Zealand White rabbits [redacted] 1987b): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge

86-3355	1	0	0	0	2	2 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
86-3358	1	0	0	0	2	2 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3359	1	0	0	0	2	2 [a]	2
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3365	1	0	0	0	2	1 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3366	1	0	0	0	2	1 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-
86-3367	1	0	0	0	1	1 [a]	1
	24	**0**	0	**0**	**0**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.00**	**0.00**	-

[a] Mucus membrane appeared blistered.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). This deviation is considered not to compromise the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited slight response in treated animals. The individual mean scores at 24, 48 and 72-hours for all animals were 0.00 for corneal opacity, iris lesions, and conjunctival chemosis and 0.33, 0.00, 0.00, 0.00, 0.00, 0.00 for conjunctival redness.

Thus, under test conditions of the study, glyphosate sodium salt is considered as not irritating to the eyes.

Assessment and conclusion by RMS:

The study is considered supplementary due to the low purity of the test substance and the limited reporting. Under the study conditions the test substance is not irritating to the rabbit eye.

B.6.2.5.24. Study 24

Data point:	CA 5.2.5/024
Report author	[redacted]
Report year	1987
Report title	Primary eye irritation study of MON-8750 in New Zealand White rabbits
Report No	[redacted]86-431/9308A
Document No	Not reported
Guidelines followed in study	US EPA 81-4 (1982)
Deviations from current test guideline (OECD 405 (2017))	No anaesthetic was given prior, during or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Clinical signs, bodyweight, sex and age of the animals, and number of air changes were not specified. The stability of the test chemical is not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting.

In an eye irritation study, six New Zealand White rabbits were treated with glyphosate (MON 8750, Batch: XLG-255, Purity: 90.8 %) by instillation of 0.1 g of the undiluted test substance into the conjunctival sac of one eye of each animal. The eyes were rinsed 24-hours after treatment with physiological saline. Following treatment, eye irritation was scored using the Draize scheme at 1, 24, 48 and 72-hours. Furthermore, the animals were observed for mortality and systemic toxicity daily. At the end of the study period, all animals were killed by intracardiac injection of sodium pentorbarbital.

Instillation of MON 8750 into the rabbit eye resulted in effects on the conjunctivae, which all resolved within 72-hours. The individual mean irritation scores (24 – 72-hours) of the rabbits were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.33, 0.67, 0.33, 0.33, 0.67, 0.33
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate is not irritating to the eye under the chosen test conditions.

Materials and methods

A: Materials

1. **Test material:**

Identification:	Glyphosate (MON 8750)
Description:	White powder
Lot/Batch #:	XLG-255
Purity:	90.8 %
Stability of test compound:	Not specified

2. **Vehicle and/ or positive control:** None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult (not further specified)
Sex:	Not specified
Initial body weight:	1.8 – 2.3 kg
Acclimation period:	At least 5 days
Diet/Food:	NIH 09 Rabbit Ration, certified feed (Zeigler Brothers, Inc., Gardners, PA, US), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire-mesh cages
Environmental conditions:	Temperature: 20 – 23 °C
	Humidity: 40 – 60 %
	Air changes: Not specified
	Photoperiod: 12 hours light / dark cycle

B: Study design and methods

In life dates: 1986-11-05 to 1986-11-05

Animal assignment and treatment:

Both eyes of all six animals were examined for lesions with fluorescein sodium and an ultraviolet lamp 24-hours before administration.

An amount of 0.1 g of the test substance was placed into the conjunctival sac of one eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. Twenty-four hours after the treatment and after scoring, the eyes were rinsed with physiological saline to remove any remaining test material.

At 1, 24, 48 and 72-hours all eyes were examined for signs of irritation. Effects on cornea, iris, and conjunctivae (redness, chemosis, and discharge) were scored using the Draize criteria. In addition, all animals were observed for mortality and systemic toxicity daily. The body weights were measured immediately prior to dosing on study day 1. All animals were euthanized by intracardiac injection of sodium pentobarbital at the end of the study period.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea an on the iris were observed in any animal. Conjunctival redness, chemosis and discharge were seen in all animals after 1 hour, however these observations resolved within 72-hours. In addition, blistering of the mucus membrane appeared blistered at 1 hour only. All effects were reversible.

A summary of the findings is given in the table below.

Table 6.2.5.17 Primary eye irritation study of MON-8750 in New Zealand White rabbits [redacted] 1987a): Eye irritation – Individual irritation scores

Animal No.	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
86-3368	1	0	0	0	1	1 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
86-3372	1	0	0	0	2	2 [a]	2
	24	**0**	0	**0**	**1**	**0**	1
	48	**0**	0	**0**	**1**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.67**	**0.00**	-
86-3372	1	0	0	0	2	2 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
86-3374	1	0	0	0	2	1 [a]	1
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-
86-3375	1	0	0	0	2	1 [a]	2
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**1**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.67**	**0.00**	-
86-3376	1	0	0	0	2	2 [a]	2
	24	**0**	0	**0**	**1**	**0**	0
	48	**0**	0	**0**	**0**	**0**	0
	72	**0**	0	**0**	**0**	**0**	0
Individual mean (24, 48, 72 h)		**0.00**	**-**	**0.00**	**0.33**	**0.00**	-

[a] Mucus membrane appeared blistered.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, the study is in concordance with the current OECD TG 405 (2017). This deviation is considered not to compromise the outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited slight response in treated animals. The individual mean scores at 24, 48 and 72-hours for the animal were 0.00 for corneal opacity, iris lesions and conjunctival chemosis, and 0.33, 0.67, 0.33, 0.33, 0.67, 0.33 for conjunctival redness.

Thus, under test conditions of the study, glyphosate is considered as not irritating to the eyes.

Assessment and conclusion by RMS:
The study is considered acceptable but with restrictions (reliable with restrictions) due to the limited reporting. Under the study conditions the test substance is not irritating to the rabbit eyes.

B.6.2.5.25. Study 25

Data point:	CA 5.2.5/018
Report author	[redacted]
Report year	1983
Report title	Mucous membrane irritation test on rabbits with glyphosate (tech) of [redacted]
Report No	Not reported
Document No	Not reported
Guidelines followed in study	None
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Environmental conditions, housing, animal age, acclimation period, and dates of the study not specified. The stability of the test chemical is not reported. Eyes were washed out after 30 seconds instead of 1 hour. Irritation was not evaluated at 1 hour. The study report is hard to read due to low quality of the pdf file and contains limited information.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	No
Acceptability/Reliability:	**Conclusion GRG:** Invalid, Category 3b **Conclusion AGG:** The study is considered unacceptable since the study is not GLP and due to the washing of the eyes and the limited reporting.

In an eye irritation study, six albino rabbits were treated with glyphosate (Batch: 8.7.83, Purity: 95 %) by instillation of 0.1 g of the undiluted test substance into the conjunctival sac of one eye of each animal. The other untreated eye served as control. The eyes of all animals were rinsed 30 seconds after treatment with water. Following treatment, eye irritation was scored using the Draize scheme at 24, 48, 72, 96 hours and at 7 and 15 days.

Instillation of glyphosate into the rabbit eye resulted in effects on the cornea, iris and conjunctivae in all animals which reversed latest by 96 hours. Though appropriate table explanations were missing in the study report, mean irritation scores (24 – 72-hours) have been derived using the standard Draize categories as follows:

- for corneal opacity: 0.33, 0.33, 0.33, 0.33, 0.00, 0.33
- for iris lesions: 0.33, 0.33, 0.33, 0.33, 0.33, 0.33
- for conjunctival redness: 1.00, 1.00, 0.67, 1.00, 0.67, 1.00
- for chemosis of the conjunctiva: 2.00, 1.33, 1.33, 1.00, 0.67, 1.00

Materials and methods

A: Materials

1. **Test material:**

Identification: Glyphosate (Tech)

Description:	White amorphous powder
Lot/Batch #:	R&D sample, sample 8.7.83
Purity:	95 %
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	NWS
Source:	[redacted]
Age:	Not specified
Sex:	Male and female
Initial body weight:	1.5 – 2.5 kg
Acclimation period:	Not specified
Diet/Food:	Lucerne grass, carrots, germinated grams with wheatbran, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually (not further specified)
Environmental conditions:	Temperature: Not specified
	Humidity: Not specified
	Air changes: Not specified
	Photoperiod: Not specified

B: Study design and methods

In life dates: Not specified

Animal assignment and treatment:

An amount of 0.1 g of the test substance was placed into the conjunctival sac of one eye of each animal (three males and three females). The other eye served as control and remained untreated. The eyes of all animals were rinsed with water 30 seconds after instillation of the test substance.

At 24, 48, 72, 96 hours, 7 and 15 days post-administration, all eyes were examined for signs of irritation. Effects on cornea, iris and conjunctivae (redness, chemosis and discharge) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

After 24-hours, effects on the cornea were observed in five of six animals, and effects on iris and conjunctivae were observed in all animals. Effects on the cornea had resolved after 48 hours while effects on the iris and conjunctivae persisted until 72-hours. After 96 hours, no effects were observed in any animal. Though

appropriate table explanations were missing in the study report, the table headings in the report have been presented in the table below according to the standard Draize categories (see table footnote). Mean values are derived from the parameters as presented here.

A summary of the findings is given in the table below.

Table 6.2.5.25-18 Mucous membrane irritation test on rabbits with glyphosate [redacted] 1983): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea [a]		Iris	Conjunctiva [b]		
		Opacity	Area		Redness	Chemosis	Discharge
Rabbit 1	**24**	**1**	1	**1**	**2**	**3**	2
	48	**0**	0	**0**	**1**	**2**	2
	72	**0**	0	**0**	**0**	**1**	1
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**2.00**	-
Rabbit 2	**24**	**1**	1	**1**	**2**	**3**	2
	48	**0**	0	**0**	**1**	**1**	2
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**1.33**	-
Rabbit 3	**24**	**1**	0	**1**	**1**	**3**	2
	48	**0**	0	**0**	**1**	**1**	1
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**0.67**	**1.33**	-
Rabbit 4	**24**	**1**	1	**1**	**2**	**2**	2
	48	**0**	0	**0**	**1**	**1**	2
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.33**	-	**0.33**	**1.00**	**1.00**	-
Rabbit 5	**24**	**0**	0	**1**	**1**	**1**	2
	48	**0**	0	**0**	**1**	**1**	1
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	-	**0.33**	**0.67**	**0.67**	-
Rabbit 6	**24**	**1**	1	**1**	**2**	**2**	3
	48	**0**	0	**0**	**1**	**1**	1
	72	**0**	0	**0**	**0**	**0**	0
	96	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0
	Day 15	0	0	0	0	0	0

Table 6.2.5.25-18 Mucous membrane irritation test on rabbits with glyphosate [redacted] 1983): Eye irritation in rabbits – Individual irritation scores

Animal	Scoring [h]	Cornea [a]		Iris	Conjunctiva [b]		
		Opacity	Area		Redness	Chemosis	Discharge
Individual mean (24, 48, 72 h)		**0.33**	**-**	**0.33**	**1.00**	**1.00**	**-**

[a] Cornea scores labelled A and B in the report. In the Draize scale for scoring ocular lesions, A is for opacity and B is for the area of cornea involved. Therefore, the column headings of "Opacity" and "Area" are used in this table for data in the report labelled A and B, respectively.

[b] Conjunctivae scores are labelled A, B, and C in the report. In the Draize scale for scoring ocular lesions, A is for redness, B is for chemosis, and C is for discharge. Therefore, the column headings of "Redness", "Chemosis", and "Discharge" are used in this table for data in the report labelled A, B, and C, respectively.

3. Assessment and conclusion

Assessment and conclusion by applicant:

Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application, removal of the test substance after 30 seconds and the missing possibitlity for calculation of individual mean scores, the study is in concordance with the current OECD TG 405 (2017).

Due to the removal of the test substance after 30 seconds and as therefore, the irritating potential of glyphosate could not be estimated, the study is considered not acceptable.

Assessment and conclusion by RMS:

Agreed with the conclusion by the applicant that the study is considered unacceptable due to the rinsing of the eye and the limited reporting.

B.6.2.5.26. Study 26

Data point:	CA 5.2.5/026
Report author	[redacted]
Report year	1981
Report title	Primary eye irritation of MON 0139 to rabbits
Report No	800260
Document No	[redacted] 80-261
Guidelines followed in study	None
Deviations from current test guideline (OECD 405, 2017)	No anaesthetic was given prior, during, or after dosing to reduce the pain. All animals were tested at the same time instead of using a tiered testing approach. Environmental conditions, housing, and diet were not specified. Test material not specified (purity, batch and stability) and eyes were washed out 20 seconds (three animals) or not rinsed (six animals) instead of 1 hour after treatment and irritation was not evaluated 1 hour post dosing. The age of the animals was not reported.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 3a **Conclusion AGG:** The study is considered unacceptable since the study is not GLP and due to the limited reporting and the missing data on the purity of the active substance tested.

In an eye irritation study, nine New Zealand White rabbits were treated with glyphosate (MON 0139, Batch: SSRT-11012, Purity: not specified) by instillation of 0.1 mL of the undiluted test substance into the conjunctival sac of the right eye of each animal. The eyes of three rabbits were rinsed 20 seconds after treatment with physiological saline. The eyes of the remaining six animals remained unwashed. Following treatment, eye irritation was scored using the Draize scheme at 24, 48 and 72-hours. A drop of fluorescein ophthalmic solution was placed onto the cornea, held there for 20 seconds and washed out with physiological saline.

Instillation of MON 0139 into the rabbit eye did not result in any effects on the cornea, iris or conjunctivae in any animal. The individual mean irritation scores (24 – 72-hours) of the rabbits (unwashed eyes) were as follows:

- for corneal opacity: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for iris lesions: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival redness: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for chemosis of the conjunctiva: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00
- for conjunctival discharge: 0.00, 0.00, 0.00, 0.00, 0.00, 0.00

Based on the study, glyphosate was not irritating to the eye under the test conditions chosen.

Materials and methods

A: Materials

1. Test material:

Identification:	Glyphosate (MON 0139), IPA salt
Description:	Amber liquid
Lot/Batch #:	SSRT-11012
Purity:	Not specified
Stability of test compound:	Not specified

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	[redacted]
Age:	Young adult
Sex:	Males and females
Initial body weight:	2.03 – 2.85 kg
Acclimation period:	At least 5 days
Diet/Food:	Not specified, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually (not further specified)
Environmental conditions:	Temperature: Not specified Humidity: Not specified Air changes: Not specified Photoperiod: Not specified

B: Study design and methods

In life dates: 1980-08-26 to 1980-08-29

Animal assignment and treatment:
Both eyes of all nine animals were examined for lesions with fluorescein sodium prior to administration of the test substance.

A volume of 0.1 mL of the test substance was placed into the conjunctival sac of the right eye of each animal. The eyelids were gently held together for one second and then released to prevent loss of the article. The left eye served as control and remained untreated. The eyes of three animals were rinsed with physiological saline 20 seconds after instillation of the test substance. The eyes of the remaining six animals were not washed.

At 24, 48 and 72-hours all eyes were examined for signs of irritation. Therefore, a drop of fluorescein sodium ophthalmic solution was placed onto the cornea for 10 seconds before it was washed out with physiological saline. Effects on cornea, iris and conjunctivae (redness, chemosis and discharge) were scored using the Draize criteria.

Results

A. MORTALITY

No mortality occurred.

B. CLINICAL OBSERVATIONS

No clinical signs of systemic toxicity were observed during the study.

C. EYE OBSERVATIONS

No effects on the cornea, iris or conjunctivae were observed in any animal at any time point.
A summary of the findings is given in the table below.

Table 6.2.5.26-19 Primary eye irritation of MON 0139 to rabbits [redacted] 1981): Eye irritation – Individual irritation scores

Animal	Scoring [h]	Cornea opacity	Iris	Conjunctiva		
				Redness	Chemosis	Discharge
Unwashed eyes						
Rabbit 1 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 2 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 3 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 4 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 5 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0

	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 6 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Washed eyes						
Rabbit 7 (male)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 8 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**
Rabbit 9 (female)	**24**	0	0	0	0	0
	48	0	0	0	0	0
	72	0	0	0	0	0
Individual mean (24, 48, 72 h)		**0.00**	**0.00**	**0.00**	**0.00**	**-**

3. Assessment and conclusion

Assessment and conclusion by applicant:
Except the deviation of no treatment with systemic analgesic or topical anaesthesia of the animals / animal eyes prior, during or after test substance application and immediate or no rinsing of the eye, the study is in concordance with the current OECD TG 405 (2017). As the lack of rinsing is considered as worst-case, this deviation is not considered to compromise the negative outcome of the study. Therefore, the study is considered acceptable and the outcome can be reported as valid.

Instillation of glyphosate into the rabbit eye elicited no response in treated animals. The individual mean scores at 24, 48 and 72-hours for all animals were 0.00 for corneal opacity, iris lesions, conjunctival redness and conjunctival chemosis.
Thus, under test conditions of the study, glyphosate is considered as non-irritating to the eyes.

Assessment and conclusion by RMS:
The study is considered unacceptable since it was not performed according to GLP. Additionally, the study report is very limited and does not contain data on the purity of the active substance. Therefore, no conclusion could be drawn.

B.6.2.6. Skin sensitization

B.6.2.6.1. Study 1

Data point:	CA 5.2.6/001
Report author	[redacted]
Report year	2011
Report title	Glyphosate technical: Local lymph node assay in the mouse
Report No	10/218-037E
Document No	Not reported

Guidelines followed in study	OECD 429 (2010)
Deviations from current test guideline (OECD 429, 2010)	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.

A sample of Glyphosate Technical (Batch: 569753; Purity: 96.3 %) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay.

The test item solutions were applied on the dorsal surface of ears of young adult, female CBA/J Rj mice (25 µL/ear) for three consecutive days (Days 1, 2, and 3). On Day 6, five hours prior to termination animals were intravenously injected via the tail vein with tritiated methyl thymidine (^{3}HTdR). Cell proliferation in the local lymph nodes was measured by incorporation of ^{3}HTdR and the values obtained were used to calculate stimulation indices (SI).

Groups of 4 mice received 50, 25 or 10 % w/v glyphosate technical in propylene glycol (PG), PG alone (negative controls) or 25 % α-Hexylcinnamaldehyde in PG (positive controls).

No mortality, systemic toxicity or local irritation was observed during the study. No treatment related effects were observed on animal body weights in any treated groups.

Stimulation index values of the test item were 1.0, 1.0 and 1.2 at treatment concentrations of 50, 25 and 10 % (w/v), respectively.

A significant lymphoproliferative response (stimulation index value of 12.2) was noted for α-Hexylcinnamaldehyde in this experiment, confirming the validity of the protocol used for this study.

Therefore, based on the results, Glyphosate Technical is not considered a skin sensitiser in the Local Lymph Node Assay.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	Dry white powder
Lot/Batch number:	569753
Purity:	96.3 % w/w
CAS#:	Not reported
Stability of test compound:	Stable under storage conditions (2 - 8 °C); expiry date: 2011-08-31

2. Vehicle and/or positive control: Propylene glycol (vehicle) / α-Hexylcinnamaldehyde (HCA) (positive control)

3. Test animals:

Species:	Mice
Strain:	CBA/J Rj
Sex:	Female, nulliparous, non-pregnant
Age:	9 - 10 weeks
Weight at dosing:	20.1 - 21.6 g
Source:	[redacted]
Housing:	Group caging in Type II. polypropylene/polycarbonate cages with

	Lignocel® Hygienic Animal Bedding and glass tunneltubes available to animals during the study	
Acclimatisation period:	13 days	
Diet:	ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, 59494 Soest, Germany, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	30 - 70 %
	Air changes:	15 – 20/hour
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In-life dates: 2010-10-20 to 2010-10-26

A sample of Glyphosate Technical (96.3 % w/w Glyphosate technical) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay, following dermal exposure when administered topically to young adult, female CBA/J Rj mice.

Animal assignment and treatment:
Vehicle selection: During the preliminary compatibility test the solubility of the test item was examined. The test item was insoluble in all solvent but formed an achievable gel-like formulation in propylene glycol (PG) and DMSO. The formulation in PG seemed more stable, therefore it was selected as vehicle for the test. The achievable maximum concentration was 50 (w/v) %.

Dose selection rationale: A Preliminary Irritation/Toxicity Test was performed on CBA/J Rj mice using two doses, at test item concentrations of 50 and 25 % (w/v), respectively. This preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 without radioactive proliferation assay.

During the Preliminary Irritation/Toxicity Test no mortality, systemic toxicity or local irritation were observed. No treatment related effect on body weights was observed. The observations recorded in this preliminary test suggest that the formulations, the application of the material and the local effects on the animal are acceptable for a valid LLNA.

Based on the results of the preliminary experiments the following dose levels were selected for the main assay: 0 (negative control), 10, 25, and 50 % (w/v) Glyphosate Technical, and positive control (25 % HCA). Each group comprised four mice.

Table B.6.2.6.1-1 Glyphosate technical: Local lymph node assay in the mouse [redacted] 2011): Animal assignment to the treatment groups

Treatment group	Test item concentration (w/v) %	Number of animals per group
Negative control (PG*)	---	4
Glyphosate Technical	50	4
Glyphosate Technical	25	4
Glyphosate Technical	10	4
Positive control (25 % HCA** in PG*)	---	4

*: propylene glycol, vehicle
**: α-Hexylcinnamaldehyde

Treatment and observations:

Each animal was topically dosed once a day for three consecutive days (Days 1, 2, and 3) on the dorsal surface

of each ear with 25 µL of the appropriate formulation, applied using a pipette. There was no treatment on Days 4 and 5.

Topical application:

All animals were observed at least once daily (Days 1 - 6) for any clinical signs, including local irritation and systemic toxicity. Individual body weights were recorded on Day 1 (beginning of the assay) and at Day 6 (prior to ^{3}HTdR injection).

Proliferation assay:

On Day 6 each mouse was intravenously injected via the tail vein with 250 µL of sterile PBS (phosphate buffered saline) containing approximately 20 µCi of ^{3}HTdR using a gauge 25G1" hypodermic needle with 1 mL sterile syringe.

Five hours after intravenous injection, the mice were sacrificed by CO_2 asphyxiation. The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps and the carcasses discarded. The nodes of mice from each test group was pooled and collected in separate Petri dishes containing a small amount (1 - 2 mL) of PBS to keep the nodes wet before processing.

A single cell suspension (SCS) of pooled lymph node cells (LNCs) were prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 mL). Pooled LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 °C. After centrifugation supernatants were discarded. Pellets were gently resuspended, and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for each group of pooled lymph nodes.

After the final washing step, the suspensions were centrifuged, and the supernatants were removed leaving a small volume (<0.5 mL) of supernatant above each pellet. Each pellet was gently agitated before suspending the LCNs in 3 mL of 5 % TCA (trichloroacetic acid) for precipitation of macromolecules. After incubation with 5 % TCA at 2-8 °C overnight (approximately 18 hours) precipitate was recovered by centrifugation at 190 x g for 10 minutes at 4 °C), and supernatants were removed, and pellets were resuspended in 1 mL of 5 % TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β-scintillation counter and ^{3}HTdR incorporation was measured for up to 10 minutes per sample.

The β-counter expressed the ^{3}HTdR incorporation as the number of radioactive disintegrations per minute (DPM). Similarly, background radiation levels were also measured in two 1 mL aliquots of 5 % TCA.

Clinical observations: During the study (Day 1 to Day 6) all animals were observed at least once daily for any clinical signs, including local irritation and systemic toxicity.

Bodyweights: The bodyweight of each animal was recorded prior to dosing on Day 1 and at Say 6 prior to injection of ^{3}H-methyl thymidine.

Statistics / Data evaluation: In the absence of any positive results, the statistical analysis of the data was not performed.

DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value ("DPM"). The results were expressed as "DPN" (DPM divided by the number of lymph nodes) following the industry standard for data presentation.

A stimulation index of 3 or greater is the criteria for defining a positive result.

The test item is regarded as a sensitiser if both of the following criteria are fulfilled:

- That exposure to at least one concentration resulted in an incorporation of ^{3}HTdR at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
- The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed in any of the treatment groups during the main study, including the positive control group.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed in any of the treatment groups during the main study, including the positive control group.

C. BODY WEIGHT

No treatment related effects were observed on body weight.

D. NECROPSY

It was not reported whether a necropsy was performed.

E. SKIN REACTIONS

No cutaneous reactions were observed at the site of the treatment in any treatment groups.

Proliferation assay: Appearance of the lymph nodes was normal in the negative control group and in the test item treated groups. Larger than normal lymph nodes were observed in the positive control group.

A significant lymphoproliferative response (stimulation index value of 12.2) was noted for HCA demonstrating the appropriate performance of the assay.

Table B.6.2.6.1-2 Glyphosate technical: Local lymph node assay in the mouse [redacted] 2011): Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical

Concentration (%w/v)	Disintegrations per minute (DPM/group)	Group DPM	Number of lymph nodes assayed	DPN	Stimulation Index Values (SI)
Background (5 (w/v) % TCA)	34	-	NA	-	-
Negative control PG	715	681	8	85.1	1.0
Glyphosate Technical 50 % in PG	717	683	8	85.4	1.0
Glyphosate Technical 25 % in PG	712	678	8	84.8	1.0
Glyphosate Technical 10 % in PG	828	794	8	99.3	1.2
Positive control 25 % HCA	8336	8302	8	1037.8	12.2

*: Propylene glycol, vehicle; **: α-Hexylcinnamaldehyde; TCA: trichloroacetic acid; N/A = not applicable

III. CONCLUSIONS

In conclusion, under the conditions of the present assay, Glyphosate Technical tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitiser) in the Local Lymph Node Assay.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 429 (2010). Therefore, the study is considered acceptable / acceptable and the outcome can be reported as valid.
After treatment of female mice with glyphosate (up to 45 %) in a local lymph node assay, stimulation index determined was less than 3-fold. Therefore, based on the results, glyphosate is not considered a skin sensitiser in the Local Lymph Node Assay.

Assessment and conclusion by RMS:
The study is considered acceptable, as it was performed under GLP and according to OECD 429 (2010). In this local lymph node assay, glyphosate technical did not show skin sensitizing potential.

B.6.2.6.2. Study 2

Data point:	CA 5.2.6/002
Report author	[redacted]
Report year	2010
Report title	Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test)
Report No	24879
Document No	NOT REPORTED
Guidelines followed in study	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), OPPTS 870.2600 (1998)
Deviations from current test guideline (OECD 406, 1992)	Temperature of 22 °C ± 3 °C was outside of the temperature set of 20 °C (± 3 °C). This deviation is not considered to affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG**: Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

Glyphosate TC (Batch: 2009051501, Purity: 96.4 %) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test.

The intradermal induction was performed with a 0.01 % dilution of the test item in physiological saline and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction was conducted for 48 hours under occlusion with the test item at 50 % one week after the intradermal induction.

Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing. The study was performed using a control group consisting of five animals, one test group consisting of ten animals, and a positive control group consisting of 20 animals.

The animals did not show any signs of systemic toxicity and showed an expected body weight development. Intracutaneous induction stage did not reveal any skin reactions. None of the vehicle control or test animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment.

Animals treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Based on the results, glyphosate TC has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	2009051501
Purity:	96.4 %
Stability of test compound:	At room temperature in the dark stable; expiry date: 2011-05-15

2. Vehicle and/or positive control: Purified water (*aqua ad iniectabilia*)

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	[redacted]
Age:	32 days
Sex:	Female
Weight at dosing:	312 - 355 g; positive control group: 249 - 317 g
Acclimation period:	At least 5 days
Diet/Food:	ssniff Ms-H V2333 (ssniff Spezialdiäten GmbH, Soest, Germany), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In pairs in Makrolon cages (MZK 80/25) with granulated textured wood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-10-15 to 2009-11-28

Animal assignment and treatment:

Glyphosate TC was tested for its sensitising effect on the skin of the young adult female Dunkin Hartley guinea pig using the Maximisation test according to Magnusson and Kligman. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals: six for the topical administration and two for the intracutaneous administration.

In the preliminary test, six concentrations of Glyphosate TC were tested by intracutaneous injection: 0.01, 0.1, 0.5, 1, 5, or l0 % suspensions in purified water. A concentration of 0.01 % revealed a discrete or patchy erythema, concentrations of 0.1 to 5 % revealed a moderate and confluent erythema, and the concentration of 10 % revealed an intense erythema and swelling 24 to 72-hours after administration, respectively.

Six concentrations of Glyphosate TC were tested by topical application: 0.5, 1, 5, 10, 25, and 50 % suspensions in purified water. No skin reactions were observed up to a concentration of 25 %. The concentration of 50 % revealed a discrete or patchy erythema 24 to 72-hours after start of exposure.

The concentrations selected for the main test were 0.01 % for the intracutaneous induction, 50 % for the topical induction, and 25 % for the challenge.

The main study was performed in ten test animals, five negative control animals, and 20 positive control animals (see table below).

Table B.6.2.6.2-1: Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) [redacted] 2010): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal	2
Epidermal	6
Main Study	
Negative Control Group (purified water)	5
Test Group	10
Positive Control Group (benzocaine)	20

The induction phase consisted of an intradermal injection at Day 0 and an epidermal application on Day 7. On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin of the shoulder region at a concentration of 0.01 % in purified water, together with injections of Freund´s Complete Adjuvant in physiological saline, or test item in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and physiological saline.

On Day 6 the skin was shaved and coated with 0.5 mL sodium laurylsulfate 10 % in vaseline in order to induce a local irritation. On Day 7 the test substance was topically applied at a concentration of 50 % in purified water to the clipped and shaved skin of the shoulder region using the patch technique. The patch was left occluded in place for 48 hours.

The challenge was conducted on Day 21 by an occlusive patch at a concentration of 25 % in purified water which was applied to the shaved and depilated left flank of each animal for 24-hours. The right flank of each animal was treated in the same way with the vehicle alone. Twenty-four (24) and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale (see table below).

Magnusson Kligman grading scale

Score	Reaction
0	No visible change
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period. No necropsy was performed.

The animals of the positive control group were treated with a 2 % benzocaine solution (dissolved in 40 % ethanolic 0.9 % NaCl solution) intracutaneously in the first induction phase and with a 5 % solution topically in the second induction phase and at challenge. The positive control was run in a separate study during May, 2009.
The vehicle control group animals were treated in the same way as the animals of the test group, but received aqua ad injectabilia instead of the test item. However, in stage 3 the left flank was treated with the test item, the right flank with the vehicle i.e. in the same way as the test group.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed.

C. BODY WEIGHT

All animals showed the expected gain in body weight.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

Intracutaneous induction did not reveal any skin reactions.

No skin reactions were observed 24 or 48 hours after the challenge treatment with glyphosate TC in the control or test group.

Animals treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1). Accordingly, the sensitivity and reliability of the experimental technique could be demonstrated.

Table B.6.2.6.2-2: Examination of Glyphosate TC in the Skin Sensitisation Test in Guinea Pigs According to Magnusson and Kligman (Maximisation Test) [redacted] 2010): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/10	0/10
Negative control group	0/5	0/5
Positive control group	20/20	20/20

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

Under the present test conditions, Glyphosate TC was found to be not sensitising to guinea pigs in a test model according to Magnusson and Kligman.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After a challenge treatment no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Based on the findings of this study, glyphosate is considered not a skin sensitizer.
The study was accepted in the previous evaluation (RAR, 2015).

B.6.2.6.3. Study 3

Data point:	CA 5.2.6/003
Report author	[redacted]

Report year	2010
Report title	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)
Report No	24607
Document No	N/A
Guidelines followed in study	OECD 406 (1992); US EPA OPPTS 870.2600 (1998); EC method B. 6. Skin Sensitisation (96/54/EC)
Deviations from current test guideline (OECD 406, 1992)	Temperature of 22 °C ± 3 °C was outside of the temperature set of 20 °C (± 3 °C). This deviation did not affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

The purpose of this study was to determine the potential of Glyphosate TC (Batch: 20090506, Purity: 97.3 %) to produce skin sensitisation reactions in guinea pigs in a test model according to Magnusson and Kligman.

A 0.5 % (0.1 mL) concentration of Glyphosate TC in purified water chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema in all ten animals 24 and 48 hours after administration. Two (2) mL of a 50 % concentration of Glyphosate TC in purified water /animal chosen for the 2nd (topical) induction stage was non-irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with 0.5 mL sodium laurylsulfate (10 %) on the day before stage 2 induction in order to induce a local irritation.

The challenge with 2 mL of a 25 % concentration of Glyphosate TC in purified water /animal revealed no skin irritation in any animal and, thus, the test item had no sensitising properties. The vehicle control revealed no skin reactions.

Animals of the same strain treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

The animals gained the expected weight within the test period. Behaviour of the animals remained unchanged. No necropsy was performed.

Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20090506
Purity:	97.3 % (Certificate of Analysis)
Stability of test compound:	No data given in the report Expiry date: May 2011

2. Vehicle and/or positive control:	Purified water (*aqua ad iniectabilia)*/ Benzocaine
3. Test animals:	
Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	32 days
Sex:	Male
Weight at dosing:	299 - 364 g (excluding positive control group) Positive control group: 319 - 346 g
Acclimation period:	At least 5 days
Diet/Food:	Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH) served as food. The food was offered, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	The animals were kept in pairs in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. BRANDENBURG, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-10-26 to 2010-01-30

Animal assignment and treatment:
The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with eight animals: six animals for the topical administration and two animals for the intracutaneous administration. Six concentrations of Glyphosate TC were tested by intracutaneous injection: 0.01, 0.1, 0.5, 1, 5, or 10 % suspensions in aqua ad iniectabilia: Concentrations up to 0.1 % did not reveal any skin reactions. Concentrations of 0.5 or 1 % revealed a discrete or patchy erythema, a concentration of 5 % a moderate and confluent erythema and a concentration of 10 % revealed an intense erythema and swelling 24 to 72-hours after administration, respectively. Six concentrations of Glyphosate TC were tested by topical application: 0.5, 1, 5, 10, 25, and 50 % suspensions in purified water. No skin reaction was observed at any concentration.

The concentrations selected for the main test were 0.5 % concentration for the 1st (intracutaneous) induction stage, a 50 % concentration for the 2nd (topical) induction stage and a 25 % concentration for the challenge. Possible sensitising properties of the test item were evaluated by administration of the test item to the shoulder region, first by intracutaneous application (stage 1) and 7 days later by topical administration (stage 2, exposure time: 48 hours).

The skin reaction results of the first induction exposure were evaluated at 24 and 48 hours, of the second induction at 48 and 72-hours after beginning of exposure.

In a challenge test (stage 3) the test item was again applied topically but to the flank region (exposure time: 24-hours). This area was then examined for reactions which might indicate sensitising properties of the test item.

Days 23 and 24: 21 hours after removing the filter paper the challenge area was cleaned and cleared of hair if necessary three hours later (at 48 hours from the start of challenge application) the skin reaction was observed and recorded. 24-hours after this observation a second observation (72-hours) was performed and recorded.

Table B.6.2.6.3-1 Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) [redacted] 2010): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	10
Negative Control Group (purified water)	5
Positive Control Group (benzocaine)	20

The skin reactions were graded according to Magnussen & Kligman.

Score	**Erythema**
0	No visible change
1	discrete or patchy erythema
2	moderate and confluent erythema
3	intense erythema and swelling

Mortality and clinical signs were recorded daily during the observation period. Body weight was determined at start of study and at study termination.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare reasons.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No necropsies were performed.

E. SKIN REACTIONS

No oedema or erythema were observed in test animals following challenge with technical glyphosate. The rate of sensitisation in the test substance treatment group was therefore 0 %.

The vehicle control revealed no skin reactions.

Animals of the same strain treated with benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Table B.6.2.6.3-2 Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) [redacted] 2010): Summary of skin reactions after challenge

Mean skin reaction scores	Intradermal induction (shoulder)		Topical induction (shoulder)		Challenge (flank)			
					48 h		72 h	
	24 h	48 h	48 h	72 h	left	right	left	right
Negative Control Group	0	0	1	1	0	0	0	0

Test Group	1	1	1	1	0	0	0	0
Positive Control Group	1	1	2	2	1	0	1	0

III. CONCLUSIONS

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Based on the findings of this study, glyphosate is considered not a skin sensitizer.
The study was accepted in the previous evaluation (RAR, 2015).

B.6.2.6.4. Study 4

Data point:	CA 5.2.6/004
Report author	[redacted]
Report year	2009
Report title	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test
Report No	C22908
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); Commission Regulation (EC) No 440/2008 (2008); method B.6
Deviations from current test guideline (OECD 406, 1992)	Temperature of 22 °C ± 3 °C was outside of the temperature set of 20 °C (± 3 °C). This deviation did not affect the study outcome. **AGG:** It is noted that no positive control group was included in the study itself, In the appendix of the report, a positive control study, conducted between Oct and Nov 2008 is described. This study was performed using alpha-hexylcinnamaldehyde and showed a positive response, showing sensitivity and reliability of the experimental technique in this laboratory.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** study is considered acceptable.

Glyphosate Technical (Batch: GI-1045, Purity: 96.66 %) was tested for its sensitising effect on the skin of 15 male Dunkin Hartley guinea pigs (ten test and five control) in the Maximisation Test. For the intradermal induction animals were injected with a 10 % dilution of the test material in purified water and in an emulsion of Freund´s Complete Adjuvant in physiological saline.

After one week, the epidermal induction phase of the study was conducted under occlusive conditions. Animals were exposed for 48 hours to a 50 % dilution of the test item in purified water. Control animals were intradermally induced with purified water and Freund´s Complete Adjuvant in physiological saline and epidermally induced with purified water under occlusive conditions.

Two weeks after epidermal induction, the control and test animals were challenged by epidermal application of the test item at 15 % in purified water and purified water alone under occlusive dressing. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

No mortality occurred during the study, no necropsies were performed. No clinical signs of systemic toxicity were observed in the animals. No positive/skin reactions (0/10 animals in the test group, 0/5 in the control group) were observed in the animals 24 and 48 hours after challenge. There was no effect on body weight gain.

Therefore, based on the results, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	GI-1045
Purity:	96.66 % w/w
Stability of test compound:	Expiry date: 2010-07 Test item dilution: Stable in purified water for 2 days

2. Vehicle and/or positive control: Purified water

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR, SPF
Source:	[redacted]
Age:	4 – 6 weeks (at pre-test / at beginning of acclimatization period)
Sex:	Male
Weight at dosing:	348 – 358 g (pre-test) 335 – 365 g (beginning of acclimation period)
Acclimation period:	Approximately 2 weeks for main study animals; no acclimation for pretest animals
Diet/Food:	Pellet standard Provimi Kliba 3418 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, 4303 Kaiseraugst, Switzerland), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type-4 cages with standard softwood bedding ("Lignocel", Schill AG, 4132 Muttenz, Switzerland)
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-01-14 to 2009-02-27

Animal assignment and treatment:

Glyphosate Technical was tested for its sensitising effect on the skin of guinea pig in the Maximization-Test according to Magnusson-Kligman. Fifteen (ten test and five control) animals were employed for the main test, and three animals for the intradermal and epidermal pretest (see table below for animal group assignments).

Table B.6.2.6.4-1: Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test (2009): Animal assignment to the treatment groups

Treatment group	Number of animals per group
Pretest	
Intradermal	1
Epidermal	2
Main Study	
Negative Control Group	5
Test Group	10

The concentrations of test substance for the main test were selected based on the results of a pre-test (during the acclimatization period of the main animals). Four intradermal injections (0.1 mL/site of a 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA)/physiological saline were made into the shaved neck of one guinea pig; six days later intradermal injections were made in the same guinea pig at concentrations of 5 %, 10 %, and 15 % of the test item in purified water; 15 % was the maximum feasible concentration due to its ability to pass through the intradermal injection needle. Dermal reactions were assessed 24-hours later. Based on the results, the test item concentration selected for the main study was 10 %.

To determine the concentration for the epidermal induction and challenge of the main study, four intradermal injections were made as described above in two guinea pigs. Six days later four patches of filter paper (3 x 3 cm) were saturated with the test item at 10 %, 15 %, 25 %, and 50 % in purified water in a volume of 0.2 mL and applied onto the clipped and shaved flanks of the same guinea pigs; 50 % was the maximum feasible concentration of the test item. Dermal reactions were assessed 24 and 48 hours after patch removal. Based on the results, the concentrations selected for the epidermal induction and challenge were 50 % and 15 %, respectively.

For the main study, the test item and vehicle were weighed, and a weight/weight dilution was prepared. Homogeneity of the test item preparation was ensured using a magnetic stirrer and/or spatula. The preparations were made immediately prior to each dosing. Homogeneity of the test item preparation was maintained during treatment using a magnetic stirrer when possible.

The intradermal induction of sensitisation in the test group was performed in the scapular region with a 10 % dilution of the test item in purified water and in an emulsion of FCA/physiological saline. The epidermal induction of sensitisation was conducted for 48 hours under occlusion with the test item at 50 % in purified in 0.3 mL water one week after the intradermal induction. The animals of the control group were intradermally induced with purified water and FCA/physiological saline and epidermally induced with purified water under occlusion.

Two weeks after epidermal induction the control and test animals were challenged by epidermal application of 0.2 mL test item at 15 % in purified water and 0.2 mL purified water alone under occlusive dressing for 24-hours. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing (according to the criteria laid down in test guidelines).

Body weights were determined at delivery/acclimatization start, at the end of the pretest, at test day 1 (day of treatment), and at the termination of the study. Mortality was checked daily. Clinical signs of toxicity were recorded daily beginning at the time of delivery and ending at study termination.

A positive control (reliability check) with a known sensitiser was conducted in a separate study from 2008-10-08 to 2008-11-14. The positive controls with α-hexylcinnamaldehyde at 3% in PEG 300 showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no treatment related deaths during the course of the study, hence no necropsies were performed. One pre-test animal was found in bad conditions before the start of pre-test and during the acclimatization period of the main test animals. This animal was sacrificed for ethical reasons and replaced by a new animal.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed in the animals.

C. BODY WEIGHT

The body weight of the animals was within the range commonly recorded for animals of this strain and age. One animal lost visible amount of body weight (31 %) before the start of the intradermal pre-test. It was killed for ethical reasons and replaced by another animal.

D. NECROPSY

No necropsies were performed.

E. SKIN REACTIONS

Skin Effects in the Intradermal Induction (Test Day 1)

The expected and common findings were observed in the control and test group after the different applications using FCA intradermally. These findings consisted of erythema, oedema, necrotizing dermatitis, encrustation, and exfoliation of encrustation.

Skin Effects in the Epidermal Induction (Test Day 8)

Control group – No erythematous or oedematous reaction was observed in the animals treated with purified water only.

Test group – Discrete/patchy erythema was observed in eight out of ten test animals at the 24-hour observation and persisted in seven animals up to the 48-hour reading after treatment with the test item at 50 % in purified water.

Skin Effects in the Challenge Procedure

Control group and Test group – No positive/skin reactions were observed in the animals when treated with either purified water only or when treated with the test item at 15 % in purified water.

Table B.6.2.6.4-2: Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test ([redacted] 2009): Summary of skin reactions after challenge

Treatment group		Incidence*	
		24-hours	48 hours
Test group	15 % Glyphosate Technical in purified water (right flank)	0/10	0/10
	Purified water (left flank)	0/10	0/10
Negative control group	15 % Glyphosate Technical in purified water (right flank)	0/5	0/5
	Purified water (left flank)	0/5	0/5

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

Based on the above mentioned findings in the Magnusson & Kligman Test in guinea pigs and in accordance to Commission Directive 2001/59/EC, Glyphosate Technical does not have to be classified and labelled as a skin sensitiser.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in any treated or control guinea pigs were observed 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
The study is considered acceptable, as it was performed under GLP and according to OECD 406. In this Maximisation study, Glyphosate technical did not show skin sensitizing potential.

B.6.2.6.5. Study 5

Data point:	CA 5.2.6/005
Report author	[redacted]
Report year	2009
Report title	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)
Report No	23915
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); US EPA OPPTS 870.2600 (1998); EC method B.6. (Skin Sensitisation)
Deviations from current test guideline (OECD 406, 1992)	Temperature of 22 °C ± 3 °C was outside of the temperature set of 20 °C (± 3 °C). This deviation did not affect the study outcome.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** Some concerns were raised regarding the work conducted at this specific testing facility. After consultation with the responsible GLP monitoring authority, no GLP not in compliance (nic) reports on studies with glyphosate by this testing facility are available or known. Therefore, the study is considered acceptable.

The potential of Glyphosate TC (Batch: 20080801, Purity: 98.8 %) to produce skin sensitisation reactions in guinea pigs was investigated in a test model according to Magnusson and Kligman.

A 0.5 % suspension of glyphosate in purified water chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema 24 and 48 hours after administration.

Two (2) mL of a 50 % suspension of Glyphosate TC in purified water/animal was chosen for the 2nd (topical) induction stage and was not irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with sodium laurylsulfate on the day before the 2nd induction in order to induce a local irritation.

The challenge with 2 mL of a 50 % suspension of Glyphosate TC in purified water/animal revealed no skin irritation in any animal and, thus, the test item had no sensitising properties.

The vehicle control revealed no skin reactions.

Animals of the same strain treated with the positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

No mortality and no clinical signs were observed. The body weight gains of the animals treated with Glyphosate TC was within the range of the vehicle control at study termination. No necropsy was performed.

Therefore, based on the results, Glyphosate TC has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate TC
Description:	White solid powder
Lot/Batch #:	20080801
Purity:	98.8 %
Stability of test compound:	No data given in the report, expiry date: 2010-08-01

2. Vehicle and/or positive control:

Vehicle:	Purified water (*Aqua ad iniectabilia*)
Positive control:	Benzocaine

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	[redacted]
Age:	32 days
Sex:	Male
Weight at dosing:	313 – 358 g (excluding positive control group); positive control group: 271 - 331 g
Acclimation period:	At least 5 days
Diet/Food:	Commercial diet, ssniffB MS-H V2233 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany), *ad libitum.*
Water:	Tap water, *ad libitum*
Housing:	In pairs, in MAKROLON cages (MZK 80/25). Granulated textured wood (Granulat A2, J. Brandenburg, 49424 Goldenstedt, Germany) was used as bedding material in the cages. The cages were changed and cleaned twice a week.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 55 ± 15 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2009-02-04 to 2009-03-28

Animal assignment and treatment:

The purpose of this study was to determine the potential of Glyphosate TC to provoke skin sensitisation reactions in guinea pigs.

A preliminary study was conducted to determine the appropriate dose level of the test item following intracutaneous and topical administration. For the intracutaneous administration, two animals were injected (scapular region, shaved and depilated to remove hair) with 0.1 mL of 0.01, 0.1, 0.5, 1, 5, or 10 % suspensions of the test item in purified water (three concentrations in each animal); 10 % was the maximum technically feasible concentration that could be administered by intradermal injection. No skin reactions were observed with the 0.01 % concentration at 24, 48, and 72-hours after administration. Discrete or patchy erythema was observed at a concentration of 0.1 % at 48 and 72-hours, and at 0.5 % at 24 to 72-hours after administration. Concentrations of 1 and 5 % revealed a moderate and confluent erythema 24 to 72-hours after administration. A concentration of 10 % revealed an intense erythema and swelling 24 to 72-hours after administration. Therefore, the concentration of 0.5 % for the intracutaneous induction of the main study.

For the topical dose selection, 2 mL of test preparation in concentrations of 0.5, 1, 10, 25, or 50 % test item in purified water was applied to the test area of three animals with shaved skin and three animals with depilated skin (two concentrations per animal); the sites were covered with an occlusive dressing for 24 or 42 hours and the application sites were assessed immediately, 42, and 48 hours (depilated) or immediately and 24-hours (non-depilated) after removal of the patch. There were no skin reactions up to the highest concentration of 50 %; therefore, 50 % was selected as the concentration for the induction and challenge of the main study.

Induction: For the main study, on Day 0 three pairs of intradermal injections of 0.1 mL were given in the shoulder region which was cleared of hair: Freund's Complete Adjuvant (FCA) diluted 1:1 with 0.9 % sodium chloride, 0.5 % test item in purified water, and 0.5 % test item in a 1:1 mixture with FCA/physiological saline. On Day 6, 0.5 mL sodium laurylsulfate 0 % in vaseline was applied to the shaved skin to induce a local irritation since Glyphosate TC was non irritating in the preliminary study. On Day 7, 50 % test item in purified water was applied to the shaved skin in the shoulder region using the patch test technique as described above for 48 hours. Skin reactions of the first induction exposure were evaluated at 24 and 48 hours, and of the second induction at 48 and 72-hours after beginning of exposure.

Challenge: On Day 21, 50 % test item in purified water was applied to the shaved skin (flank) using the patch test technique as described above for 24-hours. Skin reactions were recorded 48 and 72-hours after the start of the challenge application.

The vehicle control group were treated in the same way as the test group but received purified water instead of the test item for the induction phases. The animals were challenged with the test item in the same manner as the test group.

The positive control group were treated as described above, but with a 2 % (w/v) benzocaine in 40 % ethanolic 0.9 % sodium chloride solution intracutaneously, and with a 4 % (w/v) benzocaine in 40 % ethanolic 0.9 % sodium chloride solution topically in the induction and challenge phases. The positive control was performed in a separate study during November to December, 2008.

Animals were observed for mortality and clinical signs daily. Body weights were determined at the start and at study termination.

Table B.6.2.6.5-1: Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) [redacted] 2009): Animal assignment to the treatment groups

Group	Number of Animals Per Group
Preliminary Study	
Intracutaneous	2
Topical	6
Main Study	
Test Group	10
Negative Control Group	5
Positive Control Group (benzocaine)	20

The skin reaction to the challenge was scored according to the following criteria:

Score **Erythema (Magnusson and Kligman scale)**

0	No erythema
1	discrete or patchy erythema
2	moderate and confluent erythema
3	intense erythema and swelling

Mortality and clinical signs were recorded daily during the observation period. Body weight was determined at start of study and at study termination.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Behaviour of the animals remained unchanged. Given the negative response in all treated animals further testing was not considered necessary in order to reduce animal experiments for animal welfare reasons.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance. The significant difference in body weight between test item-treated and control animals at start of the study is regarded to be without any biological relevance.

D. NECROPSY

No necropsies were performed.

E. SKIN REACTIONS

Skin Effects in the Intradermal Induction (Test Day 1)

The expected and common findings were observed in the control and test group. These findings consisted of discrete or patchy erythema 24 and 48 hours after administration.

Skin Effects in the Epidermal Induction (Test Day 8)

There were findings of discrete or patchy erythema observed in the control and test groups, which had been treated with sodium laurylsulfate on the day before the second induction to induce a local irritation.

Skin Effects in the Challenge Procedure

No positive/skin reactions were observed in the animals when treated with either purified water only or when treated with the test item at 50 % in purified water. The rate of sensitisation in the test substance treatment group was therefore 0 %.

The positive control benzocaine in 40 % ethanolic 0.9 % NaCl solution exhibited a sensitising reaction in all animals in form of a discrete or patchy erythema (grade 1).

Table B.6.2.6.5-2: Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) [redacted] 2009): Mean skin reaction scores

Treatment groups	Intradermal induction (shoulder)		Topical induction (shoulder)		Challenge (flank) 48 h		Challenge (flank) 72 h	
	24 h	48 h	48 h	72 h	left	right	left	right
Negative Control Group	0	0	1	1	0	0	0	0

Test Group	1	1	1	1	0	0	0	0
Positive Control Group	1	1	1	1	1	0	1	0

III. CONCLUSIONS

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to Magnusson and Kligman.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
It is agreed with the applicant's conclusion. Based on the findings of this study, glyphosate is considered not a skin sensitizer.
The study was accepted in the previous evaluation (RAR, 2015).

B.6.2.6.6. Study 6

Data point:	CA 5.2.6/006
Report author	[redacted]
Report year	2009
Report title	Glyphosate – Skin Sensitization Study in Guinea Pigs. Buehler Test
Report No	12174-08
Document No	Not reported
Guidelines followed in study	US EPA OPPTS 870.2600, equivalent to OECD 406
Deviations from current test guideline (OECD 406, 1992)	Animals used as negative control were not treated at all, whereas OECD 406 requires them to be treated with the vehicle only. Evaluation of skin reactions 24 and 48 hours instead of 30 and 54 hours after challenge; humidity was in the range of 25-98 % instead of 30-70 %; It was not reported whether clinical signs of toxicity were recorded during the study. These deviations are not considered to have a significant impact on the study outcome.
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 supportive (see "assessment and conclusion by RMS" for details).

A skin sensitisation study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if test substance Glyphosate Tech Grade Mixed 5-Batch (Batch: 080704-1 thru 5, Purity: 96.4 %) produced a sensitising reaction.

Males and females were assigned to each of two groups, designated Groups I (5/sex) and II (10/sex). Group I animals remained untreated during the induction phase of the study and served as a naive control group. Group II animals, the test group, were treated with 400 mg of the test substance moistened with 2 mL of deionized water. The animals were treated once weekly for three weeks, for a total of three treatments. After a two-week rest

period, all animals (Groups I and II) were challenged at a virgin test site with an application of 400 mg of test substance moistened with 2 mL of deionized water.

The test substance produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment.

Therefore, based on the results, Glyphosate Tech Grade Mixed 5-Batch has no sensitising effect on the skin of the guinea pig in the Buehler Test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate Tech Grade Mixed 5-Batch
Description:	White powder
Lot/Batch #:	080704-1 thru 5
Purity:	96.4 % (Certificate of Analysis)
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Deionised water/ alpha-Hexylcinnamaldehyde

3. Test animals:

Species:	Guinea Pig
Strain:	Hartley-Albino
Source:	[redacted]
Age:	Approx. 4 weeks
Sex:	Male and female (nulliparous and non-pregnant)
Weight at dosing:	Male: 359-414 g; female: 341-387 g
Acclimation period:	5 days
Diet/Food:	PMI Feeds, Inc.™ Guinea Pig Diet #5025; available *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individual housing in suspended, wire bottom, stainless steel cages
Environmental conditions:	Temperature: 19 ± 2 °C Humidity: 25 - 98 % Air changes: 10 - 12/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2008-11-03 to 2009-01-02

Animal assignment and treatment:

Irritation screening was done in two male and two female animals with 400 mg of test substance moistened with deionized water, and 75 %, 50 %, and 25 % w/v concentrations of the test substance in deionized water. Based on the results of the irritation screening, Group II animals (the test group) were treated once weekly for three weeks with 400 mg of test substance moistened with 2 mL of deionized water. After a two-week rest period, all animals (Test and control) were each challenged at a virgin test site with an application of 400 mg of test substance moistened with 2 mL of deionized water. Observations for skin reactions at each test site were made approximately 24 hours after each treatment. In addition, observations for skin reactions were made approximately 48 hours after the first induction treatment and 48 hours after the challenge treatment. An average score for each time period was obtained by adding all of the scores for each time period and dividing by the number of test sites scored for that time period. The test substance is considered a sensitiser if the mean irritation

scores, the total number of animals with scores, and/or the total number of scores for the virgin test site in the test group after the challenge treatment are appreciably greater than those for the naive challenge group. The average skin reaction score of this study was 0.0.

Table B.6.2.6.6-1 Glyphosate – Skin Sensitisation Study in Guinea Pigs. Buehler Test [redacted] 2009): Animal assignment to the treatment groups

Treatment group	Number of animals (total)
Main Study	
Test Group	10/sex (20)
Negative Control Group	5/sex (10)

The skin reaction to the challenge was scored according to the following criteria:

Score	**Erythema**
0	No reaction
0.5	Very faint, usually nonconfluent
1	Faint, usually confluent
2	Moderate
3	Strong, with or without oedema

Mortality was monitored during the observation period. Body weight was determined at start of study (Day 0) and at study termination (Day 31).

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

No clinical observations were reported.

C. BODY WEIGHT

All animals gained weight during the conduct of the study.

D. NECROPSY

Performance of necropsy was not reported.

E. SKIN REACTIONS

The test substance, Glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitising reaction in guinea pigs.

A positive control was not performed within the scope of current study. However, a reliability check of the performing laboratory (with alpha-Hexylcinnamaldehyde according to the Buehler Method in guinea pigs (In life: 2008-06-05 to 2008-07-05) was provided. A mean score of 1.2 for the test group after challenge treatment, when compared with naive control group mean score of 0.1, confirmed the sensitivity of guinea pigs to the positive control material.

Table B.6.2.6.6-2 Glyphosate – Skin Sensitisation Study in Guinea Pigs. Buehler Test [redacted] 2009): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24 hours	48 hours
Test group	0/20	0/20

Negative control group	0/10	0/10

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

The test substance, Glyphosate, produced neither irritation in the test animals (Group II) nor the naive control animals (Group I) after the challenge treatment, and therefore did not elicit a sensitising reaction in guinea pigs.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with US EPA OPPTS 870.2600 guidelines, equivalent to the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
In this Buehler test, the test substance glyphosate (Tech Grade Mixed 5-Batch; Batch: 080704-1 thru 5; Purity: 96.4%) had no skin sensitising effect in guinea pigs. However, the RMS considers the study supportive only since it is generally recognised that a negative Buehler assay is less sensitive compared to an LLNA or GPMT. It is nonetheless noted that this Buehler assay shows the same results than the more sensitive assays.
This conclusion is not in line with the previous evaluation, where the study was considered acceptable (RAR, 2015).

B.6.2.6.7. Study 7

Data point:	CA 5.2.6/007
Report author	[redacted]
Report year	2008
Report title	Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test
Report No	[redacted]3996.318.431.07
Document No	Not reported
Guidelines followed in study	OECD 406
Deviations from current test guideline (OECD 406, 1992)	Animals were not weighed at the end of the test and body weights determined at the beginning of the test are not shown in the study report. A positive control group was not used; a reliability check using historical control data from the study performing laboratory was not provided. The vehicle DMSO is not the preferred one recommended by the guideline. According to the GRG, these deviations did not affect the outcome of the study.
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 not considered acceptable due to the deviations noted above. Especially the lack of a positive control and the non-provision of HCD is a major limitation of the study.

The skin sensitisation test with Glyphosate Technical (Batch: 20070606, Purity: 98.05 %) in guinea pigs (*Cavia porcellus*) was carried out according to the Buehler Test Method, in order to evaluate its skin sensitisation

potential.

A pilot study was conducted in order to determine the suitable doses of test substance for induction and challenge applications. The induction and challenge doses chosen were 0.5 g of test item (equivalent to 1 mL, of a 50 % w/v test solution). One test solution for each application was made using DMSO as vehicle to increase the contact area and to allow transdermal absorption. After each application cotton lint patches were held in contact with the skin for an approximated 6-hour exposure period. Twenty treatment animals were exposed to the test item in inductions and challenge applications. Ten control animals were exposed to the vehicle on inductions and to the test item on challenge application.

Skin reactions were evaluated approximately 30 and 54 hours after each application by clinical examination (inductions) and according to the Magnusson & Kligman's grading scale (pilot study and challenge).

Neither compound-related clinical signs nor behavioural alterations were observed during inductions. No animal from control group was positive for the test item after challenge application.

One animal from treatment group was positive for the test item after challenge application.

Therefore, based on the results, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Buehler Test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	20070606
Purity:	98.05 %
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: DMSO

3. Test animals:

Species:	Guinea Pig
Strain:	Hartley
Source:	[redacted]
Age:	Eight to nine weeks old
Sex:	Male
Weight at dosing:	444 - 556 g
Acclimation period:	7 days
Diet/Food:	Pelleted commercial diet - "Nuvilab Cobaias 6001"
Water:	Tap water (enriched with ascorbic acid (300 mg/L), *ad libitum*
Housing:	Polypropylene cages (88 x 55 x 28 cm) with autoclaved wood shavings, five animals per cage during the experimental phase
Environmental conditions:	Temperature: 18 - 23 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2008-06-12 to 2008-07-12

Animal assignment and treatment:
The skin sensitising potential of glyphosate was assessed using the Buehler Test method. The test consists of a pre-test to identify an appropriate induction and challenge concentrations of the test substance, and the Maximization test itself.

Table B.6.2.6.7-1: Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test [redacted] 2008): Animal assignment to the treatment groups

Treatment group	Number of animals
Pre-Test Study	
Pre-test Group	2
Main Study	
Test Group	20
Negative Control Group	10

The skin of the animals (left flank for inductions and right flank for challenge) was mechanically and closely clipped free of hair using an electric razor at each application day. Skin was observed for lesions after clipping the fur. According to the Buehler's application method, animals were exposed to cotton lint patches with an approximated 6 cm² surface area. To allow transdermal absorption and to increase the contact surface, the test item was applied in solution using DMSO as vehicle, according to the solubility of the test item.

Based on the preliminary study, animals of the test group of the main study were exposed to patches loaded with 1 mL of 50 % (w/v) test solutions in DMSO (equivalent 0.5 g of the test item) for the induction and challenge applications. Control animals were exposed to 1 mL of vehicle for induction and to 1 mL of the test solution during challenge. Since control animals were not exposed to the test item on inductions, a hypersensitive state could not be induced in these animals, which then constituted a negative control in order to allow the differentiation between skin irritation and skin sensitisation at challenge. Patches were held in contact with the skin by an occlusive dressing during an approximated 6-hour exposure period in each application, after which patches were carefully removed from the skin and any residue cleaned up using DMSO.

Three applications were carried out during induction phase with a seven-day interval between inductions and after a fourteen-day interval between third induction and challenge application was conducted.

Animals were clinically examined approximately 30 and 54 hours after each application. Skin reactions were evaluated in agreement with Magnusson & Kligman's grading scale after challenge application.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Neither compound-related clinical signs nor behavioural alterations were observed during inductions.

C. BODY WEIGHT

According to the study report, animals were weighed at the first day of application, however, the data are not available in the study report. It is not stated in the study report that animals were weighed at the end of the test.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

No animal from the negative control group was positive for the test item after challenge application. One animal from treatment group was positive for the test item after challenge application.

Table B.6.2.6.7-2: Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test [redacted] 2008): Summary of skin reactions after challenge

Treatment group	Incidence*	
	30 hours	54 hours
Test group	1/20	1/20
Negative control group	0/10	0/10

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

The epidermal application of Glyphosate Technical using DMSO as vehicle did not cause skin sensitisation in guinea pigs, according to the Buehler Test Method.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with OECD 406 guideline. Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate (50 %), no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
In this Buehler test, the test substance glyphosate (technical, batch: 20070606, purity: 98.05 %) had no skin sensitising effect in guinea pigs. The study, however, is not considered acceptable for evaluation due to the deviations noted above. Further, a negative Buehler assay is considered less sensitive compared to an LLNA or GPMT.
The conclusion that the study is not acceptable is not in line with the previous evaluation, where the study was considered acceptable (RAR, 2015).

B.6.2.6.8. Study 8

Data point:	CA 5.2.6/008
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test
Report No	B02316
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), JMAFF guideline 2-1-6 (2005)
Deviations from current test guideline (OECD 406, 1992)	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 to be acceptable.

For the determination of potential sensitising properties of glyphosate technical (NUP 05068) (Batch: 200609062, Purity: 95.1 %) a Maximisation Test was conducted using female guinea pig. For the main test, test-substance concentrations were selected based on the results of the pre-test. The intradermal induction was

performed with a 3 % dilution of the test item in PEG 300 and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction was conducted for 48 hours under occlusion with the test item at 50 % in PEG 300 one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing. The study was performed using one control group consisting of five females, and one test group consisting of ten females.

None of the animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment. The animals did not show any signs of systemic toxicity, and except for one animal from the pre-test, showed an expected body weight development. Alpha-hexyl¬cinnamaldehyde was used as positive control substance in a separately conducted study of the same laboratory. Skin reaction of 100 % demonstrate the sensitivity and reliability of the experimental technique.

Therefore, based on the results, glyphosate technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions (20 ± 5 °C), light protected; expiry date: 2008-09-14

2. Vehicle and/or positive control: Polyethylene glycol 300 (PEG 300)

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR, SPF
Source:	[redacted]
Age:	5 – 6 weeks
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	Pre-test: 362 – 372 g; main test: 337 – 381 g
Acclimation period:	Main test: at least 10 days
Diet/Food:	Pelleted standard Provimi Kliba 3418 guinea pig breeding / maintenance diet, containing Vitamin C (Provimi Kliba AG, CH-Kaiseraugust), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in Makrolon type 4 cages with standard softwood bedding
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 10 - 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2007-01-10 to 2007-02-15

Animal assignment and treatment:
Glyphosate technical (NUP 05068) was tested for its sensitising effect on the skin of young female Dunkin Hartley guinea pig using the Maximisation test according to Magnusson and Kligman. The test substance concentrations for the main study were selected based on the results of the pre-testing performed with three

animals. The main study was performed in ten test animals and five control animals.

Table B.6.2.6.8-1: Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test ([redacted] 2007): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	1
Epidermal Pretest	2
Main Study	
Negative Control Group	5
Test Group	10

In the pretest, four intradermal injections (0.1 mL/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant/physiological saline were made into the neck of one guinea pig. Five days later, intradermal injections (0.1 mL/site) were made at concentrations of 3, 5, and 10 % test substance in PEG 300. Dermal reactions were assessed at 24-hours. Based on the skin reactions and the ability to apply the test substance, 3 % was the concentration selected for the main study.

Also in the pretest, two guinea pigs received the intradermal injections of Freund's Complete Adjuvant/physiological saline as described above. Five days later, the test substance was applied to shaved flanks of the animals in concentrations of 10, 15, 25, and 50 % in PEG 300 in 0.2 mL or 0.2 g; 50 % was the highest feasible concentration. The patches were covered by an occlusive dressing for 24-hours. Skin reactions were assessed 24-hours after patch removal. Based on the skin reactions, 50 % and 25 % were selected as the induction and challenge concentrations, respectively.

The induction phase consisted of an intradermal injection on Day 1 and an epidermal application on Day 8. On Day 1 the test substance was injected (0.1 mL/site) into the clipped dorsal skin from the scapular region at a concentration of 3 % either in PEG 300 or in a 1:1 (v/v) mixture of Freund´s Complete Adjuvant and physiological saline. On Day 8 the test substance was topically applied at a concentration of 50 % in PEG 300 (0.3 mL of test item preparation) to the clipped and shaved skin of the scapular area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

The challenge was conducted on Day 22 by an occlusive patch containing 0.2 mL of the test material at a concentration of 25 % in PEG 300, the highest non-irritating concentration, that was applied to the clipped and shaved left flank of each animal for 24-hours. The clipped and shaved right flank of each animal was treated in the same way with the vehicle only (PEG 300). Twenty-four (24) and 48 hours after removal of the dressing skin reactions were scored according the Magnusson and Kligman grading scale.

Body weights were determined at the first day of treatment of the main study and at termination. Mortality and clinical signs were recorded daily during the study period.

A positive control (reliability check) with a known sensitiser was not included in this study. However, a separate study was performed from June to August 2006 in the laboratory. The positive controls with alpha-hexylcinnamaldehyde (3 % in PEG 300) showed that the chosen guinea pig strain was able to detect sensitising compounds under the laboratory conditions chosen.

Evaluation criteria for classification as a potential skin sensitiser: at the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed.

C. BODY WEIGHT

All animals showed the expected gain in body weight with the exception of one of the pre-test animals that did not gain body weight between the day of epidermal application and the day of sacrifice one week later.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

After induction with the test item at 50 % in PEG 300 on test day 8, discrete/patchy erythema were observed in nine out of the ten animals at the 24- and/or 48-hour reading. No erythematous or oedematous reaction was observed in the animals treated with PEG 300 only.

No skin reactions were observed 24 or 48 h after removal of the challenge treatment with glyphosate technical (NUP 05068) in the control or in the test group.

Table B.6.2.6.8-2: Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test ([redacted] 2007): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/10	0/10
Negative control group	0/5	0/5

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

According to the findings in an adjuvant sensitisation test (M&K-test) in guinea pigs, glyphosate technical (NUP 05068) is not classified for skin sensitisation based on the EU classification criteria.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction with 50 % glyphosate technical and a challenge treatment with 25 % no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance glyphosate technical (NUP 05068; batch: 200609062, purity: 95.1 %) had no skin sensitising effect in guinea pigs. The study is considered acceptable for evaluation. This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.2.6.9. Study 9

Data point:	CA 5.2.6/009
Report author	[redacted]
Report year	2007
Report title	Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse)
Report No	GM8048-REG
Document No	Not reported

Guidelines followed in study	OECD 429 (2002), OPPTS 870.2600 (2003)
Deviations from current test guideline (OECD 429, 2010)	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 to be acceptable.

A sample of Glyphosate Technical Material (Batch: 0507, Purity: 96.1 %) was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Dose levels were selected according to solubility and acute oral toxicity data (no further data were provided for the dose selection).

On three consecutive days, groups of four female CBA/Ca/Ola/Hsd mice were daily administered 25 µL of a 10, 25 or 45 % w/v preparation of the test substance in dimethyl sulphoxide to the dorsal surface of each ear. On Day 6, approximately five hours before sacrifice, all animals were injected with approximately 250 µL of phosphate buffered saline (PBS) containing 20 µCi of a 2.0 Ci/mmol specific activity ^{3}H-methyl thymidine. After sampling the draining auricular lymph nodes, a single cell suspension was prepared, and ^{3}H-methyl thymidine is measured by β-scintillation counting.

The application of the test substance at concentrations of 10, 25, and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less than 3-fold compared to the negative control at all concentrations.

In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 %, and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration compared to the negative control. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

Therefore, based on the results, Glyphosate Technical Material is not considered a skin sensitiser in the Local Lymph Node Assay.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical Material
Description:	White solid
Lot/Batch number:	0507
Purity:	96.1 % (Certificate of analysis)
Stability of test compound:	Stable under storage conditions – ambient temperature in the dark

2. Vehicle and/or positive control: Dimethyl sulphoxide and / Hexylcinnamaldehyde (vehicle:acetone in olive oil (4:1))

3. Test animals:

Species:	Mouse
Strain:	CBA/Ca/Ola/Hsd
Sex:	Female
Age:	8 - 12 weeks
Weight at dosing:	16.5 - 20.8 g
Source:	[redacted]
Housing:	Maximum four per cage, in cages suitable for animals of this strain and weight range. Environmental enrichment provided included tents, bases and nestlets.
Acclimatisation period:	At least 5 days

Diet:	Diet (RM1), supplied by Special Diets Services Limited, Witham, Essex, UK, *ad libitum*	
Water:	Mains water supplied by an automatic system, *ad libitum*	
Environmental conditions:	Temperature:	22 ± 3 °C
	Humidity:	30 - 70 %
	Air changes:	A minimum of 15 changes/hour
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In-life dates: 2007-01-10 to 2007-01-16

Animal assignment and treatment: A sample of glyphosate technical material was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The test substance was applied as 10, 25 or 45 % w/v preparations in dimethyl sulphoxide. Groups of four female CBA/Ca/Ola/Hsd mice were used for this study.

Table B.6.2.6.9-1 Glyphosate Acid: Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) ([redacted] 2007): Animal assignment to the treatment groups

Treatment group	Number of animals per group
Concentration of test substance (% w/v)	
0 (vehicle only)	4
10	4
25	4
45	4
Concentration of hexylcinnamaldehyde (% w/v)	
0 (vehicle only)	4
5	4
10	4
25	4

Dose selection rationale: Approximately 25 µL of a 10, 25, or 45 % w/v preparation of the test substance was used in this study as 45 % w/v was the limit of solubility.

Treatment preparation and administration: Approximately 25 µL of a 10, 25, or 45 % w/v preparation of the test substance in dimethyl sulphoxide was applied, using a variable volume micro-pipette, to the dorsal surface of each ear. A vehicle control group was similarly treated using dimethyl sulphoxide alone. The procedure was repeated daily for three consecutive days. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250 µL of phosphate buffered saline (PBS) containing 20 µCi of a 2.0 Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were humanely killed by inhalation of halothane vapour followed by cervical dislocation. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS.

A single cell suspension was prepared by mechanical disaggregation of lymph nodes through a 200 micron-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10 mL of PBS. Approximately 3 mL of 5 % w/v trichloroacetic acid (TCA) was added and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1 mL of TCA.

The lymph node suspensions were transferred to scintillation vials and 10 mL of scintillant (Optiphase) was added prior to β-scintillation counting using a Packard Tri-Carb 3100TR Liquid Scintillation Counter.

Clinical observations: Animals were checked at least once daily for signs of systemic toxicity.

Bodyweights: The bodyweight of each animal was recorded prior to dosing on Day 1 and prior to injection of ^{3}H-methyl thymidine on Day 6.

Positive control: The reliability of the test system was assessed in a positive control study using the same method with a known sensitiser (hexylcinnamaldehyde) applied as 5 %, 10 % or 25 % w/v preparations in acetone in olive oil (4:1). The experimental phase of the positive control study started on 19 July 2006 and was completed on 25 July 2006.

Statistics / Data Evaluation: The results are expressed as a disintegrations per minute (dpm) value per lymph node for each group. The activity of each test group is then divided by the activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration.

The criterion for a positive response is that one or more concentrations of the test substance should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit responses in standard guinea pig tests for skin sensitisation. Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a skin sensitiser.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were reported.

B. CLINICAL OBSERVATIONS

Clinical observations were made but none were reported.

C. BODY WEIGHT

Body weights were measured but no significant changes were reported.

D. NECROPSY

It was not reported whether a necropsy was performed.

E. SKIN REACTIONS

Group Mean Counts per Minute: The application of the test substance at concentrations of 10, 25 and 45 % w/v in dimethyl sulphoxide resulted in an isotope incorporation which was less than 3-fold compared to the negative controlat all concentrations. Consequently, the test substance is considered not to be a skin sensitiser under the conditions of the test.

Table B.6.2.6.9-2 Glyphosate Acid: Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) ([redacted] 2007): Radiolabel incorporation into lymph-nodes of mice treated with glyphosate technical material

Concentration of Glyphosate Technical Material (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	3912	489	N/A
10	8	2394	299	0.6
25	8	3292	412	0.8
45	8	4067	508	1.0

N/A = not applicable

In the positive control study, the application of hexylcinnamaldehyde at concentrations of 5 %, 10 % and 25 % w/v in acetone in olive oil (4:1) resulted in a greater than 3-fold increase in isotope incorporation at the 25 % w/v concentration compared to the negative control. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

Table B.6.2.6.9-3 Glyphosate Acid: Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay in the Mouse) ([redacted] 2007): Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (hexylcinnamaldehyde)

Concentration of hexylcinnamaldehyde (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test control ratio (SI)
0 (vehicle only)	8	5939	742	N/A
5	8	10111	1264	1.7
10	8	13747	1718	2.3
25	8	38015	4752	6.4

III. CONCLUSIONS

Glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 429 (2010). Therefore, the study is considered acceptable / reliable and the outcome can be reported as valid.
After treatment of female mice with glyphosate (up to 45 %) in a local lymph node assay, stimulation index determined was less than 3-fold. Therefore, based on the results, glyphosate is not considered a skin sensitiser in the Local Lymph Node Assay.

Assessment and conclusion by RMS:
Based on this LLNA test, the test substance glyphosate (technical material, batch: 0507, purity: 96.1%) is not considered a skin sensitiser. The study is considered acceptable for evaluation. This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.2.6.10. Study 10

Data point:	CA 5.2.6/010
Report author	[redacted]
Report year	2006
Report title	Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method
Report No	[redacted] 05/0218
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); 96/54/EC B.6 (1996); 12 NohSan No. 8147, Guideline No. 2-1-6 (2010)
Deviations from current test guideline (OECD 406, 1992)	It was not reported whether clinical signs of toxicity were made during the study. No data on the diet used was reported. Room temperature 19 °C – 25 °C instead of 17 °C – 23 °C. The deviations did not affect the outcome of the study.
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 to be acceptable.

The potential of Glyphosate Technical (Batch: H05H016A, Purity: 95.7 %) to produce skin sensitisation reactions in guinea pigs was investigated in a test model according to Magnusson and Kligman. Twenty test and ten control animals were used for the main study. The test-substance concentrations for the main test were selected based on the results of preliminary sighting test as follows: intradermal induction: 0.195 % (v/v) in isotonic sodium chloride solution; topical induction: 60 % (w/w) in distilled water; challenge: 60 % (w/w) and 30 % (v/v) in distilled water. Approximately 24-hours after induction as well as 24 and 48 hours after challenge, the animals were scored for skin reactions. Body weight was determined.

Two animals were found dead on Day 3 and on Day 5. No skin reactions were noted after induction and after challenge in the control group and the treatment group.

The positive response observed in the positive control validation study with alpha-Hexylcinnamaldehyde validates the test system used in the study.

Therefore, based on the results, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	H05H016A
Purity:	95.7 % (Certificate of analysis)
Stability of test compound:	No data available Expiry date: 2008-03-25

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Guinea Pigs
Strain:	Albino Dunkin Hartley
Source:	[redacted]
Age:	Not specified
Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	295 - 370 g
Acclimation period:	5 days
Diet/Food:	Not specified. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminant that could reasonably be expected to affect the purpose or integrity of the study.
Water:	Tap water, ad libitum
Housing:	Groups of two or three animals in makrolon cages furnished with woodflakes
Environmental conditions:	Temperature: 19 to 25 °C Humidity: 30 - 70 % Air changes: At least 10 changes/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 05 September 2005 to 13 October 2005

Animal assignment and treatment:

The method used for assessing the sensitising properties of the test material was based on the Guinea Pig Maximisation Test of Magnusson B & Kligman A M, (1969) J. Invest. Dermatol. 52: 268 - 276.

A preliminary study was conducted to determine the appropriate dose level of the test item following intradermal and topical administration. For the intradermal induction dose selection, injections (0.1 mL/injection site) were made on the clipped shoulder of two guinea pigs, at concentrations of 25 % (w/w), 12.5 % (v/v), 6.25 % (v/v), 3.125 % (v/v), 1.56 % (v/v), and 0.78 % (v/v) in isotonic sodium chloride solution. A macroscopic evaluation of the injection sites was conducted approximately 24-hours after injection to determine whether these concentrations caused necrosis. Due to necrosis, two additional concentrations of 0.39 % (v/v) and 0.195 % (v/v) were also investigated. The concentration chosen for use in the intradermal induction phase of the main test was 0.195 % (v/v) in isotonic sodium chloride solution since necrosis was observed at all other concentrations.

For the topical induction dose selection, two guinea pigs were treated with preparations of the test material (60 % (w/w), 30 % (v/v), 15 % (v/v), and 7.5 % (v/v) in distilled water). Applications were made to the clipped flanks under occlusive dressings for an exposure period of 24-hours. The degree of erythema and oedema was evaluated approximately 24-hours after dressing removal. The highest concentration producing only mild to moderate dermal irritation was selected for the topical induction stage of the main study, i.e. 60 %.

For the dose selection for topical challenge, four preparations of the test material (60 % (w/w), 30 % (v/v), 15 % (v/v), and 7.5 % (v/v) in distilled water) were applied to the clipped flanks of three guinea pigs under occlusive dressings for an exposure period of 24-hours. These guinea pigs did not form part of the main study but had been treated identically to the control animals of the main study, up to Day 14. The degree of erythema and oedema was evaluated approximately 24-hours after dressing removal. The highest non-irritant concentration of the test material and one lower concentration were selected for the topical challenge stage of the main study.

A group of thirty guinea pigs was used for the main study, twenty test and ten control

Table B.6.2.6.10-1 Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method ([redacted] 2006): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	20
Negative Control Group	10

Two phases were involved in the main study; (a) an induction of a response and (b) a challenge of that response. Induction of the test animals: A row of three injections (0.1 mL each) was made on each side of the spine, consisting of (a) Freund's Complete Adjuvant plus isotonic sodium chloride in the ratio 1:1, (b) a 0.195 % (v/v) formulation of the test material in isotonic sodium chloride, (c) a 0.195 % (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride.

On Day 6, the scapular region of all test and control animals was shaved and sodium lauryl sulphate (10 % in petroleum jelly) was spread evenly over the area to create local irritation. On Day 7 the same area on the shoulder region used previously for intradermal injections was treated with a topical application of the test material formulation (60 % (w/w) in distilled water) under occlusive dressing for 48 hours. The intradermal induction on the control animals was performed using an identical procedure without the test material. Injection (b) was therefore the vehicle alone, injection (c) was a 50 % formulation of the vehicle in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. Similarly, the topical induction procedure was identical to that used for the test animals except that the test material was omitted.

For the challenge phase, test material formulation at the maximum non- irritant concentration (60 % (w/w) in distilled water) was applied to one side of the shorn flank of each animal under an occlusive dressing. To ensure that the maximum non-irritant concentration was used at challenge, the test material at a concentration of 30 % (v/v) in distilled water was similarly applied under an occlusive dressing to the opposite skin site on the shorn flank. After 24-hours, the dressing was carefully removed and discarded. The topical challenge sites were cleaned if required. Prior to the 24-hour observation the flanks were clipped to remove regrown hair.

Approximately 24 and 48 hours after challenge dressing removal, the degree of erythema and oedema was

quantified. Any other reactions were also recorded.

The skin reaction to the challenge was scored according to the following criteria:

Scales for Evaluation of Skin Reactions

Score	**Erythema**
0	No visible modification
1	Slight or patches of erythema
2	Moderate confluent erythema
3	Intense erythema and swelling
Score	**Oedema**
0	No visible modification
1	Slight oedema
2	Moderate oedema
3	Severe oedema

II. RESULTS AND DISCUSSION

A. MORTALITY

One test group animal was found dead on Day 3 and one other test group animal was found dead on Day 5. The cause of death was not determined but was considered not to be treatment related. The absence of these animals was considered not to affect the purpose or integrity of the study.

B. CLINICAL OBSERVATIONS

Clinical observations were not reported.

C. BODY WEIGHT

Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

No necropsy was performed.

E. SKIN REACTIONS

No skin reactions were noted in treated animals after the topical induction phase or topical challenge phase (see table below).

The historical positive control alpha-hexylcinnamaldehyde was reported to exhibit a sensitising reaction in all animals after 24-hours at a concentration of 25 and 50%.

Table B.6.2.6.10-2 Glyphosate Technical: Skin Sensitisation in the Guinea Pig – Magnusson and Kligman Maximisation method ([redacted] 2006): Summary of skin reactions after challenge

Treatment group	**Incidence***			
	30 % **		**60 %**	
	24 h	**48 h**	**24 h**	**48 h**
Test Group	0/20	0/20	0/20	0/20
Negative Control Group	0/10	0/10	0/10	0/10

*: Number of animals with findings / number of animals tested
**: Test substance concentration during challenge phase

III. CONCLUSIONS

The test material produced a 0 % (0/18) sensitisation rate and was considered as non-sensitiser to guinea pig skin under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant: I
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate (30 and 60 %), no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance glyphosate technical (Batch: H05H016A, Purity: 95.7 %) had no skin sensitising effect in guinea pigs. The study is considered acceptable for evaluation. This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.2.6.11. Study 11

Data point:	CA 5.2.6/011
Report author	[redacted]
Report year	2005
Report title	Glyphosate acid technical – Dermal Sensitization in Guinea Pigs (Buehler Method)
Report No	15279
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); US EPA OPPTS 870.2600 (2003); JMAFF 59 NohSan No. 4200 (1985)
Deviations from current test guideline (OECD 406, 1992)	Yes, humidity was not provided. It was not reported whether clinical signs of toxicity were recorded during the study. Animals used as negative control were not treated at all, whereas OECD 406 requires them to be treated with the vehicle only.
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 (see "assessment and conclusion by RMS" for details).
Category study in AIR 5 dossier (L docs)	Category 2a

A dermal sensitisation test was conducted with guinea pigs to determine the potential for Glyphosate Acid Technical (Batch: 040205, Purity: 97.23 %) to produce sensitisation after repeated topical applications.

Four-hundred milligram of a 70 % w/w mixture of the test substance in distilled water was topically applied to twenty healthy test guinea pigs, once each week for a three-week induction period. Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (HNIC, determined in the preliminary irritation screen to be a 70 % w/w mixture in distilled water) was applied to a naive site on each guinea pig. A naive control group (ten animals) was maintained under the same environmental conditions and treated with the test substance at challenge only. Approximately 24 and 48 hours after each

induction and challenge dose, the animals were scored for erythema.

The positive response observed in the historical positive control validation study with alpha-Hexylcinnamaldehyde Technical (HCA) validates the test system used in this study.

Therefore, based on the results, Glyphosate Acid Technical has no sensitising effect on the skin of the guinea pig in the Buehler Test.

I. MATERIALS AND METHODS

A. Materials

1. Test material:

Identification:	Glyphosate Acid Technical
Description:	White crystalline powder
Lot/Batch #:	040205
Purity:	97.23 % (Certificate of analysis)
Stability of test compound:	No data given in the report

2. Vehicle and/or positive control: Distilled water/ alpha-Hexylcinnamaldehyde Technical

3. Test animals:

Species:	Guinea pig
Strain:	Hartley albino
Source:	[redacted]
Age:	Young adult
Sex:	Male and Female
Weight at dosing:	327-391 g
Acclimation period:	5 or 38 days
Diet/Food:	Pelleted Purina Guinea Pig Chow #5025
Water:	Tap water, *ad libitum*
Housing:	The animals were group housed in suspended stainless steel caging with mesh floors or plastic perforated bottom caging
Environmental conditions:	Temperature: 18-22 °C Humidity: Not reported Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 2004-05-03 to 2004-06-03

Animal assignment and treatment:

A dermal sensitisation test was conducted with guinea pigs to determine the potential for Glyphosate Acid Technical to produce sensitisation after repeated topical applications. The study consists of a preliminary test to identify an appropriate induction and challenge concentrations of the test substance, and main test.

Table B.6.2.6.11-1: Glyphosate acid technical – Dermal Sensitisation in Guinea Pigs (Buehler Method) [redacted] 2005): Animal assignment to the treatment groups

Treatment group	Number of animals
Pre-Test Study	

Pre-test Group	4
Main Study	
Test Group	20
Negative Control Group	10

On the day before application of the test substance, the fur of the animals was removed by clipping the dorsal area and flanks.

Four-hundred milligram of a 70 % w/w mixture of the test substance in distilled water was topically applied to twenty healthy test guinea pigs, once each week for a three-week induction period animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed, and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings of local reactions (erythema) according to the scoring system was performed.

Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (determined in a preliminary irritation test with 4 animals to be a 70 % w/w mixture in distilled water) was applied to a naive site on each guinea pig. A naive control group (ten animals) was maintained under the same environmental conditions and treated with the test substance at challenge only. Approximately 24 and 48 hours after each induction and challenge dose, the animals were scored for erythema.

The skin reaction to the challenge was scored according to the following criteria:

Scoring System

Score	**Erythema**
0	No reaction
0.5	Very faint erythema, usually non-confluent
1	Faint erythema, usually confluent
2	Moderate erythema
3	Severe erythema with or without oedema

Individual body weights of the animals were recorded prior to initiation and again on the day after challenge.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

Clinical observations were not reported.

C. BODY WEIGHT

Body weight was recorded but an analysis of the data was not performed.

D. NECROPSY

Performance of necropsy was not reported.

E. SKIN REACTIONS

Induction Phase:
Induction treatment with 70 % w/w mixture of the test substance in distilled water caused very faint erythema (0.5) at most test sites.

Challenge Phase:
After challenge with 70 % w/w mixture of the test substance in distilled water very faint erythema (0.5) was observed at six of twenty test sites 24-hours following the challenge application. Similar irritation persisted at one affected site through 48 hours.

Very faint erythema (0.5) was also noted at two of ten naive control sites 24 hours following the challenge application. All control animals were free of irritation by 48 hours.

The positive response observed in the historical positive control validation study with HCA validates the test system used in the study. After induction very faint to faint erythema (0.5-1) was noted for all positive control sites during the induction phase. After challenge seven of ten positive control animals exhibited signs of a sensitisation response (faint erythema) 24-hours after challenge. Similar indications persisted at three of these sites through 48 hours.

Table B.6.2.6.11-2: Glyphosate acid technical – Dermal Sensitisation in Guinea Pigs (Buehler Method [redacted] 2005f): Sensitisation response indices

	Incidence of Positive Response		Severity	
	24 h	**48 h**	**24 h**	**48 h**
Test Group	6/20	1/20	0.15	0.025
Negative Control Group	2/10	0/10	0.10	0.00

III. CONCLUSIONS

Based on these findings and on the evaluation system used, Glyphosate Acid Technical is not considered to be a contact sensitiser.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with OECD 406 guidelines. Therefore, the study is considered acceptable and the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
A Buehler assay in guinea pigs was performed with 70% (w/w) glyphosate acid (Technical, Batch: 040205, Purity: 97.23%) to investigate its skin sensitising properties. The applicant, as well as the study author, concluded that no skin reactions were observed and that the test item is not a skin sensitiser in this assay.

The RMS, however, considers the study results to be equivocal. Faint erythema were observed in 6/20 and 1/20 test animals after 24 and 48 hours, respectively. The severity of these reactions was scored as '0.5' in all animals. According to OECD 406, animals should be scored with whole numbers only. Therefore, it is debatable whether the severity of the skin reactions should instead be scored as '1'. In Table B.6.2.6.11-2, the severity is scored under consideration of a score '0.5' for the six animals nonetheless. In case a score '1' would be used, numbers would double. The RMS recognises that also some animals from the negative control group showed positive responses at 24 hours, (all scored with '0.5' as well) but not at 48 hours. However, the incidence is lower compared to the test group so that the incidence in the test group still offsets the incidence of the negative control group.

According to the CLP regulation, the test substance should be classified as skin sensitiser (sub-category 1B) since $\geq$ 15% of the animals respond to a topical induction dose of > 20% in this Buehler assay. As stated before, however, the results are rather equivocal. A weight-of evidence approach with regard to skin sensitising properties of the active substance is performed in Volume 1.

Lastly it is noted that it is generally recognised that Buehler assays are less sensitive compared to an LLNA or GPMT, i.e. negative results from a Buehler assay should be considered with caution. A positive Buehler assay, in contrast, is considered to be acceptable.

The conclusion made by the RMS is not in line with the previous evaluation in which the study was fully accepted and the test item was not considered a skin sensitiser (RAR, 2015).

B.6.2.6.12. Study 12

Data point:	CA 5.2.6/012
Report author	[redacted]
Report year	1996
Report title	Glyphosate Acid: Skin Sensitisation To The Guinea Pig
Report No	[redacted] P/4699
Document No	Not reported
Guidelines followed in study	OECD 406 (1992); 92/69/EC B.6 (1992); US EPA Guidelines Section 81-6
Deviations from current test guideline (OECD 406, 1992)	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 to be acceptable.

In a dermal sensitisation study with glyphosate acid (Batch: P24, Purity: 95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman (1970). The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response. The sensitisation response of the animals was determined 24 and 48 hours after challenge by assessing the degree of erythema. The dose levels selected for the induction and challenge stages of the study were determined by a sighting study in the guinea pig.

In the main study, a 0.1 % w/v preparation in deionised water was used for the intradermal injections and a 75 % w/v preparation of glyphosate acid in deionised water was used for the topical application. For challenge concentrations of 75 and 30 % (w/v) preparation of glyphosate acid in deionised water were used.

Challenge of previously-induced guinea pigs with a 75 % w/v preparation of glyphosate acid in deionised water elicited a response characteristic of an irritant.

Challenge of previously-induced guinea pigs with a 30 % w/v preparation of glyphosate acid in deionised water did not elicit a skin sensitisation response.

A historical positive control study using hexylcinnamaldehyde demonstrated the sensitivity of the test system.

Therefore, based on the results, glyphosate acid has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch number:	P24
Purity:	As given in report 95.6 % a.i
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Deionised water and / hexylcinnamaaldehyde (HCA)

3. Test animals:

Species:	Guinea pig
Strain:	Albino Cr1 (HA) BR
Age:	Young adult
Sex:	Female
Weight at dosing:	250 - 317 g
Source:	[redacted]
Housing:	Individually in suspended cages in racks suitable for animals of this strain and the weight range expected during the course of the study.
Acclimatisation period:	At least 6 days
Diet:	RGP), supplied by Labsure, Manea, Cambridgeshire, UK, *ad libitum*
Water:	Mains water, *ad libitum*
Environmental conditions:	Temperature: 17 ± 2 °C
	Humidity: 40 - 70 %
	Air changes: Approximately 25 changes/hour
	Photocycle: 12 hours light/dark cycle

B: Study design and methods

In-life dates: 1995-04-25 to 1995-05-19

Animal assignment and treatment: In a dermal sensitisation study with glyphosate acid (95.6 % w/w a.i.) young adult, female, Albino Cr1 (HA) BR guinea pigs were tested using the method of Magnusson and Kligman (1970). The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response. Doses were selected according to results obtained from a sighting study.

Table B.6.2.6.12-1 Glyphosate Acid: Skin Sensitisation to the Guinea Pig [redacted] 1996): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test group – treated with test substance at both the induction and challenge	20
Negative control group – treated with test substance only at the challenge	10

Induction: An area approximately 5 x 5 cm on the scapular region of each animal was clipped free of hair and a row of three injections (0.05 - 0.1 mL each) was made on each side of the mid-line. The injections were:

i) Top: Freund's Complete Adjuvant plus deionised water in the ratio 1:1;
ii) Middle: a 0.1 % w/v preparation of the test substance in deionised water;
iii) Bottom: a 0.1 % w/v preparation of the test substance in a 1:1 preparation of Freund's Complete Adjuvant plus deionised water.

Control animals were treated the same as the test animals, except that they were treated with deionised water in place of the test substance.

One day prior to topical induction, the application site was clipped and 0.5 mL of a 10 % w/v preparation of sodium lauryl sulphate in paraffin wax was applied in order to provoke a mild inflammatory response.

One week after intradermal injection, the scapular area was treated with a topical application of the test substance as a 75 % w/v preparation in deionised water. This preparation (0.2 - 0.3 mL) was applied on filter paper (approximate size 4 cm x 2 cm) which was held in place by a piece of surgical tape. The tape was covered by a strip of adhesive bandage (approximate size 20 – 30 cm x 5 cm) and secured by a piece of self-adhesive PVC tape. This occlusive dressing was kept in place for approximately 2 days.

Deionised water only was applied to the filter paper for control animals.

The application sites were checked approximately 1 day after removal of the dressings.

Challenge: Two weeks after the topical inductions, an area, approximately 15 cm x 5 cm, on both flanks of all the test and control animals, was clipped free of hair. An occlusive dressing was prepared which consisted of two pieces of filter paper (approximate size 1 cm x 1.5-2.0 cm) stitched to a piece of rubber sheeting (approximate size 12 cm x 5 cm).

A 75 % w/v preparation of the test substance in deionised water (0.05 - 0.1 mL) was applied to one of the pieces of filter paper and a 30 % w/v preparation in deionised water (0.05 - 0.1 mL) was applied to the second piece of filter paper. The dressing was placed on the shorn flank of the guinea pig so that the 75 % w/v preparation was on the left and the 30 % w/v preparation was on the right. It was then covered with a strip of adhesive bandage (approximate size 25-40 cm x 7.5 cm) which was secured by a self-adhesive PVC tape.

After approximately 1 day, the dressings were carefully removed. Skin sites were examined approximately 1 and 2 days after removal of the dressings and any erythematous reactions were quantified and recorded, using a four-point scale.

Score	Dermal Observations
0	No reaction
1	Scattered mild redness
2	Moderate diffuse redness
3	Intense redness and swelling

Positive Controls: The sensitising potential of hexylcinnamaldehyde (HCA) was assessed essentially as described above to demonstrate the sensitivity of the strain of animals used and the reliability of the experimental technique. A concentration of 0.3 % w/v HCA in corn oil was used for the intradermal injections and HCA was used undiluted for the topical induction and challenge applications.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities were reported.

B. CLINICAL OBSERVATIONS

Clinical observations were made but none were reported.

C. BODY WEIGHT

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

D. NECROPSY

There was no necroscopy performed at the end of the study.

E. SKIN REACTIONS

Induction reactions and duration: Not reported.

Challenge reactions and duration: Following challenge of previously-induced guinea pigs with a 75 % w/v preparation of the glyphosate acid in deionised water, scattered mild redness (score ‘1’) was seen in three of the twenty test animals and one of the ten control animals. This response is considered to be due to skin irritation following topical challenge. The basis for this conclusion is that an equivalent reaction (score ‘1’) was seen in one of the ten control animals and the reaction was restricted to the 24-hour clinical observation only, which is characteristic of a mild skin irritation reaction rather than skin sensitisation.

Following challenge of previously-induced guinea pigs with a 30 % w/v preparation of the glyphosate acid in deionised water, no reaction was seen in any of the test or control animals. The net percentage response was

calculated to be 0 %.

Positive control: Following challenge of previously induced guinea pigs, scattered mild redness or moderate diffuse redness was observed in 14/20 test animals. Scattered mild redness was seen in two of the ten control animals. The net % response was 50 % and, therefore, HCA was classified as a moderate skin sensitiser which demonstrated the sensitivity of the strain of animals used and the reliability of the experimental technique.

Table B.6.2.6.12-2 Glyphosate Acid: Skin Sensitisation to the Guinea Pig [redacted] 1996): Summary of skin reactions after challenge

Treatment group	Incidence*			
	75 %**		30 %**	
	24-hours	48 hours	24-hours	48 hours
Main test – test group	3/20	0/20	0/20	0/20
Main test – negative vehicle control	1/10	0/10	0/10	0/10
	Incidence* (100 %**)			
	24-hours		48 hours	
Positive control – test group	14/20		13/20	
Positive control – vehicle control	2/10		0/10	

*: Number of animals with findings / number of animals tested
**: Test substance concentration during challenge phase

III. CONCLUSIONS

Glyphosate acid is not a skin sensitiser under the conditions of the test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable / reliable and the outcome can be reported as valid.
After challenge treatment with glyphosate (30 %), no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance glyphosate acid (batch: P24, purity: 95.6 % w/w) had no skin sensitising effect in guinea pigs. The study is considered acceptable for evaluation. This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.2.6.13. Study 13

Data point:	CA 5.2.6/013
Report author	[redacted]
Report year	1995
Report title	HR-001: Dermal sensitisation study in Guinea pigs
Report No	[redacted]95-0036
Document No	Not reported
Guidelines followed in study	OECD 406 (1992), US EPA FIFRA Guideline Subdivision F (1984)
Deviations from current test guideline (OECD 406, 1992)	Not reported whether clinical signs of toxicity were recorded.
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 to be acceptable but with restrictions (reliable with restrictions) (see 'assessment an conclusion by RMS' for details).

For the determination of potential sensitising properties of HR-001 (Glyphosate Technical, Batch: T-941209; Purity: 97.56 %), the test item was applied to the skin of female Hartley strain guinea pig. DNCB (2,4-dinitro-chlorobenzene) was used as the positive control substance. Animals were assigned to the following four groups:

- 20 animals to the test substance treatment group, treated with the test substance both at the induction and challenge,
- 20 animals to the negative control group for the test substance, treated with the test substance at the challenge but not at the induction,
- Ten animals to the DNCB treatment group treated with DNCB both at induction and challenge, and
- Ten animals to the negative control group for DNCB, treated with DNCB at the challenge but not at the induction.

Concentrations of 5 %, 25 %, and 25 % of the test substance, and concentrations of 0.1 %, 1 %, and 0.5 % of DNCB were selected as the dose for intradermal induction, epidermal induction, and epidermal challenge, respectively. Skin reactions to the challenge were observed 24 and 48 hours after removal of the patch and dermal sensitisation rates were calculated.

All 20 animals in the test substance treatment group exhibited the reaction of 0 (no reaction).

All 20 animals in the negative control group for the test substance also exhibited score 0. Thus the sensitising rate, i.e. the percentage of animals positively sensitised, was 0 % in the test substance treatment group.

The positive control group administered DNCB had nine animals because one animal died at Day 12 after intra-dermal induction. These animals exhibited a reaction of score 3 (intense redness and swelling). All ten animals in the negative control group for DNCB exhibited a score of 0. Thus the sensitising rate of DNCB was greater than 100 %. This was sufficient to assure the reliability of this study.

Therefore, based on the results, HR-001 (Glyphosate Technical) has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	HR-001 (Glyphosate technical)
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56 %
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Paraffin oil (intradermal); white petrolatum (epidermal) and DNCB (2,4-dinitrochlorobenzene as positive control.

3. Test animals:

Species:	Guinea pig
Strain:	Crj:Hartley
Source:	[redacted]
Age:	6 weeks
Sex:	Female
Weight at dosing:	332 – 423 g

Acclimation period:	8 days	
Diet/Food:	Pellet diet GC4 (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, Japan), *ad libitum*	
Water:	Filtered and sterilized water, *ad libitum*	
Housing:	Aluminium cage with wire-mesh floor, 5 animals/cage	
Environmental conditions:	Temperature:	23.9 – 24.0 °C
	Humidity:	51.8 - 56.3 %
	Air changes:	15/hour
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In life dates: 1995-04-19 to 1995-05-13

Animal assignment and treatment:
The test was carried out according to the Maximisation method of Magnusson and Kligman.

A preliminary test was performed to determine the dose levels for intradermal injections and topical exposure. Intradermal injections were administered to four animals at dose levels of 0.5 %, 1 %, 2.5 %, and 5 % (w/v) of the test substance in Freund's Complete Adjuvant (FCA) in sterilized physiological salt solution (SPS). The test substance caused moderate and diffuse redness or weaker irritation at all concentrations.

To determine the epidermal induction and challenge doses, four animals received doses of 10 % and 25 %, and another four animals received doses of 15 % and 20 %; the test substance was applied in a mixture with white petrolatum by a closed patch application. None of the concentrations caused any irritation to the skin.

Based on these results, twenty female Hartley guinea pigs (Crj:Hartley) were exposed to concentrations of 5 %, 25 %, and 25 % glyphosate technical for intradermal induction, epidermal induction, and epidermal challenge, respectively.

The positive control, 2,4-dichlorobenzene (DCNB), was administered to ten females at concentrations of 0.1 %, 1 %, and 0.5 % for intradermal induction, epidermal induction, and epidermal challenge, respectively.

Groups of ten and 20 animals were used for the negative control group for DCNB (treated with DCNB at the challenge but not at the induction) and the negative control group for technical glyphosate (treated with test substance at the challenge but not at the induction), respectively.

Table B.6.2.6.13-1 HR-001: Dermal sensitisation study in Guinea pigs [redacted] 1995): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test group – treated with test substance at both the induction and challenge	20
Negative control group – treated with test substance only at the challenge	20
Positive control group- treated with DNCB at both the induction and challenge	10
Negative control group for DNCB – treated with DNCB only at the challenge	10

For the main test, animals in the test and positive control groups received three pairs of intradermal injections: 0.1 mL water in oil emulsion of FCA blended with SPS, 0.1 mL test substance or DNCB in paraffin oil, and 0.1 mL test substance or DNCB in FCA blended with SPS. Animals in the negative control groups received similar injections without test substance or DNCB.

On Day 6 after the intradermal induction, 10 % sodium lauryl sulfate in white petrolatum was applied to the shaved skin by open application. On Day 7 after the intradermal induction, the animals in the test and positive

control groups were exposed to the test substance and DNCP, respectively; 0.4 g of the preparation was applied to filter paper (2 cm x 4 cm) which was applied to the skin and covered with an occlusive dressing for 48 hours. The negative control groups were treated in a similar way, but without the test substance or DNCB.

On Day 13 after the topical induction, the test and negative control groups were topically exposed to 0.4 g of the test substance as described above for 24-hours. Animals in the positive control and negative control group for DNCB were exposed to 0.4 g DNCB as described above.

Skin reactions to the challenge were recorded 24 and 48 hours after removal of the patch, and dermal sensitisation rates were calculated as [(No. of animals positively sensitised)/(No. of animals examined) x 100]. The skin reaction to the challenge was scored according to the following criteria:

Score	**Reaction**
0	No reaction
1	Scattered mild redness
2	Moderate and diffuse redness
3	Intense redness and swelling

Body weights were measured at the first induction and 48 hours after the removal of the patch. Necropsy was conducted for one animal of the DNCB treatment group that died on Day 12.

II. RESULTS AND DISCUSSION

A. MORTALITY

One animal died in the DNCB treatment group on Day 12 after the intradermal induction. The remaining animals in this group did not show any abnormality in the health condition and the skin reactions were clearly observed.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were reported.

C. BODY WEIGHT

No abnormal body weight changes were noted in any animal of the four groups.

D. NECROPSY

One animal died in the DNCB treatment group. At necropsy of the dead animal, consolidation of lung and hydrothorax were noted. These findings were associated with the hindrance of circulation and the respiratory abnormality, which led to the death.

E. SKIN REACTIONS

No data were provided on skin irritations after induction.

Following challenge with 25 % technical glyphosate, no oedema or erythema were observed in test animals. The rate of sensitisation in the test substance treatment group was therefore 0 %.

The rate of sensitisation in the DNCB treatment group was 100 %, which was considered to sufficiently assure the reliability of this study.

Table B.6.2.6.13-2: HR-001: Dermal sensitisation study in Guinea pigs [redacted] 1995): Summary of skin reactions after challenge

Treatment group	**Incidence***			
	No reaction	**Scattered mild redness**	**Moderate and diffuse redness**	**Intense redness and swelling**

	Score 0	1	2	3
Test group – treated with test substance at both the induction and challenge	20/20	0/20	0/20	0/20
Negative control group – treated with test substance only at the challenge	20/20	0/20	0/20	0/20
Positive control group- treated with DNCB at both the induction and challenge	0/10	0/10	0/10	9/10
Negative control group for DNCB – treated with DNCB only at the challenge	10/10	0/10	0/10	0/10

*: number of animals with skin reaction / number of animals tested

III. CONCLUSIONS

Based on the results, it was concluded that glyphosate technical (HR-001) had no dermal sensitising potential in the guinea pig Maximisation test.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction and a challenge treatment with glyphosate technical no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance HR-001 (glyphosate technical, batch: T 941209; purity: 97.56 %) had no skin sensitising effect in guinea pigs. The study is considered acceptable but with restrictions (reliable with restrictions) since the RMS considers the dose at topical/epidermal induction (25%) not sufficiently high. Since no skin reactions were noticed in the sighting study at 25%, it would have been possible to test higher concentrations in the sighting study up to concentrations that cause mild to moderate skin irritation. This conclusion is not completely in line with the previous evaluation where the study was considered fully acceptable (RAR, 2015).

B.6.2.6.14. Study 14

1. Information on the study

Data point:	CA 5.2.6/014
Report author	[redacted]
Report year	1994
Report title	Glyphosate: Magnusson & Kligman maximisation study in the guinea pig.
Report No	710/19
Document No	Not reported
Guidelines followed in study	OECD 406 (1992)
GLP	Yes
Previous evaluation	Not part of the evaluation in the previous RAR (2015)
Short description of study design and observations:	A guinea pig maximisation test was performed with ten test and five negative control animals (female; Dunkin-Hartley). Glyphosate technical (batch not reported; purity 95%) was used as test item, which was mixed with arachis/peanut oil before dosing. Test animals were dosed intradermally with 1% (w/v) and topically with 50% (w/w) during the induction phase. At challenge, animals were dosed with 50 and 25% (w/w) of the test item. Doses were selected based on the results of a sighting test.
Short description of	After intradermal dosing, all test and control animals showed some

results:	skin reactions. After topical induction, all animals showed some skin reactions after one hour, but not after 24 hours. Control animals did not show any skin reactions after topical induction. No skin reactions were noted after the challenge dose. The test material was considered not sensitizing. Historical positive control data are summarised in the study report and are considered acceptable. The reliability was sufficiently demonstrated.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Conclusion GRG: A full study report to evaluate was not available. Category 4a Conclusion AGG: In contrast to the applicant, the AGG has access to the study report and the concise summary above was written by the AGG. The study was adequately performed. The only significant deviation that was noted was the missing batch specification of the test item. The study is therefore considered acceptable but with restrictions (reliable with restrictions). Based on this GPMT, the test item is not considered a skin sensitiser.
Reasons why the study report is not available for submission	Conclusion GRG: The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL Conclusion AGG: See above.

B.6.2.6.15. Study 15

1. Information on the study

Data point:	CA 5.2.6/015
Report author	[redacted]
Report year	1994
Report title	Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig
Report No	545/42
Document No	Not reported
Guidelines followed in study	US EPA Guidelines Section 81-6 (largely in line with OECD 406)
Deviations from current test guideline (OECD 406, 1992)	A positive control was not included in the study; nevertheless historical control data not older than 6 months were provided. It was not reported whether clinical signs of toxicity were measured.
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 to be acceptable.

Glyphosate premix (Batch: 290-Jak-146-4; Purity: 62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 25 % dilution of the test item in distilled water and an emulsion of Freund's Complete Adjuvant (FCA)/distilled water. The epidermal induction was conducted under occlusion with

undiluted test material one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of undiluted test material under occlusive dressing.

The study was performed using one control group consisting of ten animals, and one test group consisting of 20 animals. None of the animals exhibited a positive skin reaction after the challenge treatment. There was no effect on body weight gain.

Historical control data confirm the sensitivity and reliability of the experimental technique within six months compared to the current study. Therefore, based on the results, glyphosate premix has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate premix
Description:	Pale yellow liquid
Lot/Batch #:	290-Jak-146-4
Purity:	62.2 % as glyphosate isopropylamine salt; 46.1 % as glyphosate
Stability of test compound:	Not reported; expiry date: 1995-09-30

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	8 to 12 weeks old
Sex:	Female
Weight at dosing:	340 - 450 g
Acclimation period:	At least 5 days
Diet/Food:	Guinea Pig FDI Diet, supplied by Special Diet Services Limited, Witham, Essex, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed 5 test animals/cage; all 6 control animals were housed together
Environmental conditions:	Temperature: 21 - 24 °C Humidity: 47 - 67 % Air changes: 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1994-03-22 to 1994-05-02

Animal assignment and treatment:
Glyphosate premix was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman.
The concentrations to be used at each stage of the main study were determined by 'sighting tests' in which four guinea pigs were intradermally injected with preparations of test material (1%, 5%, 10% or 25% w/v in distilled water), two guinea pigs were treated with the undiluted test material and three preparations of test material (75%, 50% and 25% v/v distilled water) and two guinea pigs were exposed to three preparations of the test material

(75%, 50% and 255 v/v in distilled water) to the flanks under occlusive dressing for a period of 24 hours.
Female Dunkin Hartley guinea pigs with body weights ranging from 340 to 450 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals and 10 control animals.

Table B.6.2.6.15-1 Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig [redacted] 1994): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	4
Epidermal Pretest	2
Main Study	
Negative Control Group	10
Test Group	20

The induction phase consisted of a row of 3 injections that were made on each side of the mid-line. The injections (0.1 mL/site) consisted of Freund´s Complete Adjuvant plus distilled water in the ratio of 1:1; a 25 % w/v dilution of test material in distilled water; and a 25 % w/v dilution of test material in 1:1 preparation of Freund's Complete Adjuvant plus distilled water.

One week after (Day 7) the injection phase, an epidermal application was made. Animals were clipped again, and undiluted test material was topically applied to the same shoulder area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 1 and 24 hours after removal of the bandage.

Two weeks after the topical indication, test and control animals were challenged with an occlusive patch containing 0.1 to 0.2 mL of undiluted test material. To ensure that the maximum non-irritant concentration was used at the challenge, the test material at a concentration of 75 % v/v in distilled water was applied similarly applied to a separate skin site on the right shorn flank. Vehicle was applied alone to the left shorn flank. Approximately 24 and 48 hours after the challenge, the degree of erythema and oedema was quantified.

Control animals were administered intradermal injections using the same procedure noted above for the test animals except the injections were as follows: Freund´s Complete Adjuvant plus distilled water in the ratio of 1:1; distilled water; and Freund´s Complete Adjuvant plus distilled water in the ratio of 1:1. Topical applications used the same procedures as those noted for test animals except that the vehicle alone was applied.

No positive control animals were evaluated during the study. Historical control data were provided in which different positive controls were tested. The reliability of the test system was adequately demonstrated.

Body weights were recorded at the start of the main test and on test completion.

Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were not reported.

C. BODY WEIGHT

Body weights and body weight gains of guinea pigs in the test group, between Day 0 and Day 24, were comparable to those of the control animals.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

Skin reactions after topical induction: Very slight erythema with or without very slight oedema was noted at the induction site of nine test group animals at the l-hour observation. Very slight erythema persisted at the induction sites of four test group animals at the 24-hour observation. No skin reactions were noted at the treatment sites of control group animals at the 1 and 24-hour observations.

Skin reactions after challenge: No skin reactions were observed 24 or 48 hours after the challenge treatment with glyphosate premix in the control or test group.

Table B.6.2.6.15-2 Glyphosate Premix: Magnusson & Kligman Maximisation Study in the Guinea Pig [redacted] 1994): Summary of skin reactions after challenge

Treatment group	Incidence*	
	24-hours	48 hours
Test group – undiluted	0/20	0/20
Test group – 75%	0/20	0/20
Negative control group	0/10	0/10

*: number of animals with findings / number of animals tested

III. CONCLUSIONS

Based on the results of the study, glyphosate premix is not considered a skin sensitiser.

Assessment and conclusion by applicant:
Except to the deviation of providing no control group, the GLP study is in concordance with the current OECD TG 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction with 25 % glyphosate technical and a challenge treatment with 75 % no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance glyphosate premix (Batch: 290-Jak-146-4; Purity: 62.2% as glyphosate isopropylamine salt; 46.1% as glyphosate) had no skin sensitising effect in guinea pigs. The study is considered acceptable for evaluation. This conclusion is in line with the previous evaluation (RAR, 2015).

B.6.2.6.16. Study 16

1. Information on the study

Data point:	CA 5.2.6/016
Report author	[redacted]
Report year	1994
Report title	Glyphosate [redacted]). Sensitisation in Guinea Pigs
Report No	[redacted]94-406/G
Document No	Not reported
Guidelines followed in study	OECD 406 (1981)
Deviations from current test	Yes, 8 instead of a minimum of 20 animals used in the treatment group;

guideline (OECD 406, 1992)	exposure per induction event and for the challenge for 30 seconds instead of 6 hours; evaluation of skin reactions after 24, 48 and 72-hours instead of 30 and 54 hours after challenge; age of animals not reported; one out of two persons responsible for animal keeping did not sign the study report; except of body weight, no individual animal data provided and therefore, no scores on skin reaction of single animals were reported; experimental procedures are only briefly described; no pilot study was conducted and therefore no conclusion on induction and challenge concentrations to be used in the test were provided; the highest concentration to cause mild irritation for induction and the maximum non-irritant concentration for challenge were not determined; it is not known whether a negative control group has been included; limited data on the historical positive control DNCB (2,4-dinitro-chlorobenzene) which is not one of the preferred substances as recommended in the guideline.
Previous evaluation	According to the applicant, the study was accepted during the previous evaluation (RAR, 2015). The RMS, however, was not able to retrieve this study in the previous RAR.
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Conclusion GRG: Supportive, Category 2a Conclusion AGG: The study is not considered acceptable for evaluation due to the multiple deviations noted above.

Glyphosate (Batch: 36300892; Purity: 97.2 %) was tested for its sensitising effect on the skin of the guinea pig using the modified Buehler test method. During the induction phase, eight male animals were treated with 0.5 mL of an aqueous solution of 1.2 % glyphosate; it was not reported whether a negative control group was evaluated. For the positive control group, results from historical positive control animals treated with dinitrochlorobenzene were used. Applications were made for six hours per day, three days per week, ten times altogether. Two weeks after the final induction animals were challenged with the glyphosate solution. Dermal irritation was scored at 24, 48, and 72-hours after the challenge application.

All animals survived until study termination. There were no body weight changes that were considered treatment-related. The general health of the treated animals was unaffected by treatment.

The combined indices for erythema and oedema were 0.00, 0.00, and 0.00 for the 24, 48, and 72-hour observation periods, respectively, after challenge. No individual animal data on skin reactions were provided.

For the historical positive control group, the combined indices obtained at hours 24, 48, and 72 were 3.83, 3.00, and 1.50, respectively, corresponding to moderate, moderate, and slight sensitisation.

Therefore, based on the results, glyphosate is a non-sensitising substance in guinea pigs.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White or almost white crystalline powder
Lot/Batch #:	36300892
Purity:	97.2 %
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Water / 1-chloro-2,4-dinitrobenzene (DNCB)

3. Test animals:

Species:	Guinea pig
Strain:	Albino
Source:	[redacted]
Age:	Not reported
Sex:	Male
Weight at dosing:	Not reported
Acclimation period:	It was reported that animals were quarantined but the duration was not reported.
Diet/Food:	Standard guinea pig chow supplemented with carrots, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: 20 ± 2 °C Humidity: 45 – 70 % Air changes: 10/hour Photocycle: Not reported

B: Study design and methods

In life dates: 1994-01-17 to 1994-02-23

Animal assignment and treatment:
Glyphosate was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler test method. It was not reported how the test substance concentrations for the main study were selected. The main study was performed in eight test animals. It was not reported whether negative control animals were evaluated. For the positive control group, results from historical positive control animals treated with dinitrochlorobenzene were used.

Table B.6.2.6.16-1: Glyphosate ([redacted]). Sensitisation in Guinea Pigs [redacted] 1994): Animal assignment to the treatment groups

Treatment group	Number of animals
Main Study	
Test Group	8

One day before the first induction, the hair was mechanically removed from the flanks of each guinea pig. The next day, 0.5 mL of the test material in a 1.2 % solution in water was applied by intense rubbing for 30 seconds. The induction was performed a total of ten occasions at two day intervals on the right and left flank, respectively. Animals were inspected over the next days for signs of primary irritancy. The applicant stated in its study summary that "*after each administration, an occlusive dressing was used to hold the patch in place for six hours.*" The RMS, however, was not able to retrieve this information from the study report. Therefore, it is assumed that animals were exposed only for 30 seconds per treatment.

Two weeks after the last exposure, animals were challenged with the test material. The challenge exposure was performed similarly as the induction exposures. Subsequent skin reactions were scored for both local oedema and erythema at hours 24, 48, 72, and 96. The skin reaction after the challenge was scored according to the to the Draize's method.

Clinical signs of toxicity were observed and recorded. Body weights were determined prior to treatment and weekly thereafter for five weeks.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred in during the study period.

B. CLINICAL OBSERVATIONS

No signs of toxicity were observed.

C. BODY WEIGHT

Body weights were unaffected by treatment.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

The combined indices for erythema and oedema were 0.00, 0.00, and 0.00 for the 24, 48, and 72-hour observation periods, respectively, after challenge with glyphosate. For the historical positive control group, the combined indices obtained at hours 24, 48, and 72 were 3.83, 3.00, and 1.50, respectively, corresponding to moderate, moderate, and slight sensitisation. No individual scores were reported.

III. CONCLUSIONS

Based on the results of the study, 1.2 % glyphosate solution in water applied epicutaneously on the guinea pig skin proved to be non-sensitising agent.

Assessment and conclusion

Assessment and conclusion by applicant
The GLP study is in concordance with OECD TG 406 (1981). Therefore, the study is considered acceptable and valid. Nevertheless, due to the deviations from the current OECD TG 406 (1992), such as reduced test animal number, no justification of concentration, no individual skin scores provided and limited reporting, the study can be used as supplementary information, only.
After challenge treatment with 1.2 % glyphosate, no skin reactions in guinea pigs were observed after 24, 48 and 72-hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the modified Buehler Test.

Assessment and conclusion by RMS:
The study is not considered acceptable due to the multiple deviations noted above; therefore, no statement about the skin sensitising potential of the test item is made solely based on this study. The RMS was not able to retrieve this study in the previous evaluation (RAR, 2015).

B.6.2.6.17. Study 17

1. Information on the study

Data point:	CA 5.2.6/017
Report author	[redacted]
Report year	1993
Report title	Skin sensitization test in guinea pigs with glyphosate technical 95 % of [redacted]
Report No	[redacted] 1230
Document No	Not reported
Guidelines followed in study	OECD 406 (1981)
GLP	Yes
Previous evaluation	Previously submitted (accepted in Monograph, B5, 2000, not re-evaluated in RAR Vol 3, B6, 2015)
Short description of	In a guinea pig maximisation study, 20 English guinea pigs (sex

study design and observations:	unknown) were induced intradermally with 5% of the test material (glyphosate technical, batch not reported, purity 95% min.) in propylene glycol and topically with 50% of the test material in petrolatum. For challenge, animals were exposed to 50% of the test material in petrolatum. Doses were selected based on a range-finding study. In addition to the treatment group, 20 animals (sex unknown) were included in a negative control group.
Short description of results:	All treated animals were normal and did not show any toxic symptoms. No animal died during the conduct of the study. Body weight gain was found to be normal. The test material was considered not sensitizing since no dermal reactions were noted after challenge. No concurrent or historical positive control data are available. The reliability of the study results cannot be ascertained.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Conclusion GRG: A full study report to evaluate was not available. Category 4a Conclusion AGG: In contrast to the applicant, the AGG has access to the study report and the summary written above was prepared by the AGG. The study was adequately performed and was largely in line with OECD 406. The only significant deviation that was noted was the missing batch specification of the test item and no data regarding concurrent or historical positive control data. The study is therefore considered acceptable but with restrictions (reliable with restrictions). Based in this GPMT, the test item is not considered a skin sensitiser.
Reasons why the study report is not available for submission	Conclusion GRG: The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL. Conclusion AGG: See above.

B.6.2.6.18. Study 18

1. Information on the study

Data point:	CA 5.2.6/018
Report author	[redacted]
Report year	1992
Report title	Dermal Sensitization Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design – (EPA-OECD-EEC-MAFF)
Report No	3044.229
Document No	Not reported
Guidelines followed in study	US EPA Guidelines Section 81-6 (1984); OECD 406 (1987)
Deviations from current test guideline (OECD 406, 1992)	Yes, 10 instead of a minimum of 20 animals used in the treatment group, control animals were not treated with the vehicle during induction; evaluation of skin reactions 24 and 48 hours instead of 30 and 54 hours after challenge; environmental conditions for animals not fully reported (missing temperature and humidity data); age of animals at study initiation not reported; purity of the test substance not provided; clinical signs of toxicity not reported; DNCB (2,4-dinitro¬chlorobenzene) used as positive control is not one of the preferred substances recommended in the guideline.
Previous evaluation	According to the applicant, the study was accepted during the previous evaluation (RAR, 2015). The RMS, however, was not able to retrieve this study in the previous RAR.

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: The study is not considered acceptable due to the multiple deviations noted above.

For the determination of potential sensitising properties of MON 8722 (glyphosate, sodium salt, Batch: RUD-9108-3241-F; Purity: not reported) a modified Buehler test was conducted. The test-substance concentrations for the main test were selected based on the results of the pre-test. The epidermal induction was performed with undiluted, moistened test item under occlusive conditions and was repeated once per week for a total of three applications. Fourteen (14) days after induction the animals were challenged by epidermal application of undiluted test material, moistened with saline, under occlusive dressing.

The study was performed using five male and five female Dunkin Hartley albino guinea pigs for the control and the test group, respectively. None of the animals exhibited a positive skin reaction after the challenge treatment.

DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance in a separately conducted study of the same laboratory. These data demonstrate the susceptibility of guinea pigs to sensitisation.

Therefore, based on the results of this study, MON 8722 (glyphosate, sodium salt) has no sensitising effect on the skin of the guinea pig in the modified Buehler Test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 8722 (Glyphosate, sodium salt)
Description:	White granular powder
Lot/Batch #:	RUD-9108-3241-F
Purity:	Not reported
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Distilled water / dinitrochlorobenzene historical control data

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	Not reported
Sex:	Male and female
Weight at dosing:	Male: 356 - 393 g; females: 334 – 391 g
Acclimation period:	At least 5 days
Diet/Food:	Agway Prolab Guinea Pig formula provided *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1991-08-13 to 1991-10-30

Animal assignment and treatment:

MON 8722 was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler test method. The test substance concentrations for the main study were selected based on the results of the pre-testing. In the preliminary test, four animals were treated topically with undiluted test material (100 %) and with concentrations of 50 %, 25 %, and 10 % w/v of the MON 8722 in distilled water. Based on results of the pre-test, the undiluted material was found to be non-irritating and was, therefore, administered at 100 % concentration for both induction and challenge. The main study was performed using five animals per sex for the test and the control group, respectively.

Table B.6.2.6.18-1 Dermal Sensitisation Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design [redacted], 1992): Animal assignment to the treatment groups

Treatment group	Number of animals	
Pretest	**Males**	**Females**
Epidermal Pretest	2	2
Main Study	**Males**	**Females**
Test Group	5	5
Challenge Irritation Control	5	5
Rechallenge Irritation Control*	5	5

* A rechallenge irritation control group was maintained on the study, however, the rechallenge procedure was not required/conducted.

One day prior to the induction phase, the hair was clipped from the left side of five male and five female test animals. The next day, the test site was moistened with distilled water and 0.4 g of undiluted test substance was applied to the test site. The exposure occurred under occlusive conditions. Approximately six hours after dosing, test sites were wiped with gauze moistened with distilled water. This induction procedure was repeated once per week for a total of three applications. Following each induction, test sites were scored for dermal irritation. Following the induction phase, the test animals were left untreated for a period of 14 days. Challenge and rechallenge controls animals remained untreated throughout the induction phase.

On the day prior to challenge hair was clipped from the posterior left side of the test animals and from the ten previously untreated guinea pigs. Test sites were moistened with distilled water and 0.4 g of undiluted MON 8722 was applied to the test area. An occlusive dressing was then placed over the test site. After six hours of exposure, the test site was wiped with gauze moistened with distilled water. Twenty hours after patch removal, residual hair was removed with a commercial depilatory. Test sites were graded for dermal irritation at 24 and 48 hours.

Historical control data from animals exposed to dinitrochlorobenzene was used as positive control.

All animals were observed daily for viability and weekly for clinical signs of toxicity. Body weights were recorded at the start of the main test and on test completion.

Any animal showing erythema (score 1 or higher) at the site of challenge was considered to have shown a positive response. Scores of ± (barely perceptible erythema) were considered equivocal.

The skin reaction to the challenge was scored according to the following criteria:

Score	**Dermal Observations**
0	No reaction
±	Slight patchy erythema
1	Slight, but confluent or moderate patchy erythema
2	Moderate confluent erythema

3 Severe erythema with or without oedema

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were not reported.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No dermal irritation was observed during the induction phase. No skin reactions were observed 24 or 48 hours after the challenge treatment with MON 8722 in the control or test group.

Table B.6.2.6.18-2 Dermal Sensitisation Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design [redacted] 1992): Summary of skin reactions after challenge

Treatment group / animal number	Skin reaction (scores)	
	24-hours	48 hours
Test group – undiluted		
Male 1	0	0
Male 2	0	0
Male 3	0	0
Male 4	0	0
Male 5	0	0
Female 1	0	0
Female 2	±	0
Female 3	±	±
Female 4	±	0
Female 5	±	0
Mean*	0.2	0.1
Challenge irritation controls		
Male 6	0	0
Male 7	±	0
Male 8	0	0
Male 9	0	0
Male 10	0	0
Female 6	0	0
Female 7	0	0
Female 8	0	0
Female 9	0	0
Female 10	0	0

Table B.6.2.6.18-2 Dermal Sensitisation Study in Guinea Pigs with MON 8722 – Modified Buehler Study Design ([redacted] 1992): Summary of skin reactions after challenge

Mean*	0.1	0.0

* For the purpose of calculation, equivocal results (±) are counted as 0.5.

III. CONCLUSIONS

Under the conditions of the study, MON 8722 exhibited no potential to induce dermal sensitisation in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with Guidelines Section 81-6, equivalent to the current OECD 406 (1992). Due to the deviations from the current OECD TG 406 (1992), such as reduced test animal number, no reporting of the test substance purity, the study can be used as supplementary information, only.
After challenge treatment with MON 8722, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the modified Buehler Test.

Assessment and conclusion by RMS:
The study is not considered acceptable due to the multiple deviations noted above. Nevertheless, it is noted that equivocal dermal reactions were reported for treated and control animals, with the incidence being higher in the treatment group (4/10 compared to 1/10 in the control group). The severity of these reactions was scored as ±. According to OECD 406, animals should be scored with whole numbers only. Therefore, it is debatable whether the severity of the skin reactions should instead be scored as '1'. In Table B.6.2.6.18-2, the severity is scored under consideration of a score '0.5' for the four animals nonetheless. In case a score '1' would be used, numbers would double. The RMS recognises that also one animal from the negative control group showed positive responses at 24 hours (scored with '0.5' as well) but not at 48 hours. However, the incidence is lower compared to the test group so that the incidence in the test group still offsets the incidence of the negative control group.

According to the CLP regulation, the test substance should be classified as skin sensitiser (sub-category 1B) since ≥ 15% of the animals respond to a topical induction dose of > 20% in this Buehler assay. As stated before, however, the results are rather equivocal. A weight-of evidence approach with regard to skin sensitising properties of the active substance is performed in Volume 1.

The RMS was not able to retrieve this study in the previous evaluation (RAR, 2015).

B.6.2.6.19. Study 19

Data point:	CA 5.2.6/019
Report author	[redacted]
Report year	1991
Report title	[redacted] Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig
Report No	349/11
Document No	Not reported
Guidelines followed in study	OECD 406 (1981); Commission Directive 84/449/EEC B.6
Deviations from current test guideline (OECD 406, 1992)	Batch and purity of the test substance not provided; clinical signs of toxicity not recorded; low temperature 15-22 °C instead of 20 °C (± 3 °C); humidity of 26-64 % instead of 30-70 %; positive HCD were slightly older than required by OECD 406 (approx. 9 months instead of 6 months).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Conclusion GRG: Valid, Category 2a Conclusion AGG: The study is considered acceptable but with restrictions (reliable with restrictions) due to the deviations noted above.

Glyphosate technical (Batch and Purity: not reported) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 0.1 % dilution of the test item in distilled water and an emulsion of Freund's Complete Adjuvant (FCA)/distilled water. The epidermal induction was conducted under occlusion with the test item at 50 % one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing.

The study was performed using one control group consisting of ten animals, and one test group consisting of 20 animals. None of the animals exhibited a positive skin reaction after the challenge treatment. There was no effect on body weight gain.

Formaldehyde was used as the positive control substance in a separately conducted study of the same laboratory. These data demonstrate the susceptibility of guinea pigs to sensitisation. Therefore, based on the results of this study, Glyphosate Technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	[redacted] GLYPHOSATE TECHN.
Description:	White powder
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

2. Vehicle and/or positive control:

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin-Hartley
Source:	[redacted]
Age:	8 - 12 weeks old
Sex:	Female
Weight at dosing:	328 - 440 g
Acclimation period:	At least 5 days
Diet/Food:	Guinea Pig FDI Diet, Special Diet Services Limited, Witham, Essex, U.K., *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed in groups up to 3 animals/cage in solid-floor polypropylene cages furnished with softwood shavings
Environmental conditions:	Temperature: 15 - 22 °C Humidity: 26 - 64 % Air changes: 15/hour Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1990-12-12 to 1991-02-03

Animal assignment and treatment:

Glyphosate technical was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals and ten control animals.

Table B.6.2.6.19-1 [redacted] Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted] 1991): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	4
Epidermal Pretest	2
Main Study	
Negative Control Group	10
Test Group	20

The induction phase consisted of a row of three injections made of each side of the mid-line on Day 0. One week later an epidermal application was made on Day 7.

On Day 0 the test substance was injected (0.1 mL/site) into the clipped dorsal skin from the shoulder region as: Freund´s Complete Adjuvant and distilled water in a 1:1 ratio; a 0.1 % (w/v) dilution of test material in distilled water; or a 0.1 % (w/v) dilution of test material in a 1:1 preparation of Freund´s Complete Adjuvant and distilled water. On Day 7 the test material was topically applied at a concentration of 50 % to the same shoulder area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

The challenge was conducted on Day 21 by an occlusive patch containing 0.1 to 0.2 mL of the test material at a concentration of 25 % w/w in distilled water that was applied to the shorn right flank of each animal for 24-hours. The vehicle alone was similarly applied to the left shorn flank.

Control animals were administered intradermal injections using the same procedure noted above for the test animals except the injections were as follows: Freund´s Complete Adjuvant and distilled water in a 1:1 ratio; distilled water; or Freund´s Complete Adjuvant and distilled water in a 1:1 ratio. Topical applications used the same procedures as those noted for test animals except that the vehicle alone was applied to filter paper. No positive control animals were evaluated.

Body weights were determined at Day 0 through Day 24.

Evaluation criteria for classification as a potential skin sensitiser:

At the 24-hour and/or 48-hour reading, 30 % or more of the test animals exhibit a positive response (scores ≥ 1) in the absence of similar results in the vehicle control group.

The skin reaction to the challenge was scored according to the following criteria:

Score	**Dermal Observations**
0	No reaction
1	Scattered mild redness
2	Moderate and diffuse redness
3	Intense redness and swelling

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was reported.

B. CLINICAL OBSERVATIONS

The study report did not provide clinical observations.

C. BODY WEIGHT

All animals showed the expected gain in body weight throughout the study.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate technical (NUP 05068) in the control or test group.
The known contact sensitiser, formaldehyde, produced an 87 % (20/23) sensitisation rate. This was considered to be a satisfactory sensitisation response for this material under the conditions of the test.

Table B.6.2.6.19-2 [redacted] Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig ([redacted] 1991): Skin reactions after challenge**

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/20	0/20
Negative control group	0/10	0/10

*: Number of animals with findings / number of animals tested
**: Challenge concentration: 25 %

III. CONCLUSIONS

Based on the results of the study, glyphosate is not a skin sensitiser.

Assessment and conclusion by applicant:
Except to deviations, such as no purity reported, the GLP study is in concordance with OECD 406 (1992). Therefore, the study is considered acceptable and reliable, the outcome can be reported as valid.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance glyphosate technical (batch and purity not reported) had no skin sensitising effect in guinea pigs. Due to some deviations from the guideline, however, the study is considered acceptable but with restrictions (reliable with restrictions). In the previous evaluation, the study was considered acceptable (RAR, 2015).

B.6.2.6.20. Study 20

Data point:	CA 5.2.6/020
Report author	[redacted]
Report year	1989
Report title	Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the

	Guinea Pig
Report No	5887
Document No	Not reported
Guidelines followed in study	OECD 406 (1981); Commission Directive 84/449/EEC B.6; OPPTS 870.2600
Deviations from current test guideline (OECD 406, 1992)	Purity not given (but assumed to be 98.6%, see below); no detailed data on positive control group animals provided; mean humidity reported, only; air change per hour 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

Glyphosate technical (Batch: 206-Jak-25-1, Purity: not reported but assumed to be 98.6%, see details below) was tested for its sensitising effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 10 % dilution of the test item in distilled water and an emulsion of Freund's Complete Adjuvant (FCA)/distilled water. The epidermal induction was conducted under occlusion with the test item at 25 % one week after the intradermal induction. Two weeks after induction the animals were challenged by epidermal application of the test item at 25 % under occlusive dressing.

The study was performed using one control group consisting of 20 animals, and one test group consisting of 20 animals. None of the animals exhibited a positive skin reaction after the challenge treatment. There was no effect on body weight gain.

The positive control, 2,4-dinitro-chlorobenzene (DNCB) revealed 100 % positive skin reactions in the positive control group animals.

Therefore, based on the results, glyphosate technical has no sensitising effect on the skin of the guinea pig in the Maximisation test.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification: Glyphosate technical

Description: White powder

Lot/Batch #: 206-Jak-25-1

Purity: Batch 206-JaK-25-1 reported with 98.6 %, see [redacted] 1991 (see CA 5.2.1/025)

Stability of test compound: Not reported

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species: Guinea pig

Strain: Albino Dunkin-Hartley

Source: [redacted]

Age: Young adults (less than 1 year old)

Sex:	Female (nulliparous and non-pregnant)
Weight at dosing:	302 - 466 g
Acclimation period:	7 days
Diet/Food:	FDI Guinea Pig Diet, supplied by Special Diet Services Limited, Witham, Essex, *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Housed five test animals/cage; all six control animals were housed together
Environmental conditions:	Temperature: 19 - 21 °C Humidity: 53 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1989-06-14 to 1989-07-17

Animal assignment and treatment:

Glyphosate technical was tested for its sensitising effect on the skin of the guinea pig using the Maximisation test according to Magnusson and Kligman. Female Dunkin Hartley guinea pigs with body weights ranging from 302 to 466 g were used. The test substance concentrations for the main study were selected based on the results of the pre-testing. The main study was performed in 20 test animals and 20 control animals.

Table B.6.2.6.20-1 Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig [redacted] 1989): Animal assignment to the treatment groups

Treatment group	Number of animals
Pretest	
Intradermal Pretest	2
Epidermal Pretest	2
Main Study	
Negative Control Group	20
Test Group	20

The induction phase consisted of six injections to the scapular region, three in a row made on each side of the mid-line. The anterior and middle injections (0.1 mL/site) consisted of Freund´s Complete Adjuvant (anterior) and a test material (middle). The posterior injection (0.5 mL/site) consisted for the test material emulsified in Freund´s Complete Adjuvant. The test material was injected at a concentration of 10 % (w/v) in distilled water.

Six days after the injection phase, an epidermal application was made. Animals were re-shaved and wetted with a 10 % aqueous solution of sodium lauryl sulphate to provoke mild inflammation. After 24-hours, the test material was topically applied at a concentration of 25 % to the same shoulder area and covered with an occlusive dressing, which was left in place for 48 hours. The reaction sites were assessed 24 and 48 hours after removal of the bandage.

Two weeks after the topical indication, test and control animals were challenged with an occlusive patch containing the test material at a concentration of 25 %.

Control animals were administered intradermal injections using the same procedure noted above for the test animals except the injections were with vehicle (i.e., distilled water). Topical applications used the same procedures as those noted for test animals except that the vehicle alone was applied. No positive control animals were evaluated.

The sensitivity of the strain of guinea pig to a known sensitiser, 2,4-dinitro-chlorobenzene (DNCB), was checked at six month intervals. The most recent positive control test with DNCB was completed on 1988-12-16.

Body weights were recorded at the start of the main test and on test completion.

Any animal showing erythema at the site of challenge was considered to have shown a positive response.

II. RESULTS AND DISCUSSION

A. MORTALITY

One control animal died during the study. No further information is provided in the study report on the death of this animal.

B. CLINICAL OBSERVATIONS

No clinical signs, other than skin reactions induced by treatment, were noted.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

After induction treatment, slight or discrete erythema (score 1) was observed 1 and 24-hours after treatment.

No skin reactions were observed 24 or 48 h after the challenge treatment with glyphosate technical in the control or test group.

After treatment of guinea pigs with the positive control, DNCB 100 % of the test group animals reacted positively. No individual animal data were reported.

Table B.6.2.6.20-2 Glyphosate Tech.: Magnusson & Kligman Maximisation Study in the Guinea Pig [redacted] 1989): Summary of skin reactions after challenge**

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/20	0/20
Negative control group	0/19	0/19

*: Number of animals with findings / number of animals tested
**: Challenge concentration: 25 %

III. CONCLUSIONS

There is no evidence from the test results, that Glyphosate Technical is a sensitiser in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with the current OECD 406 (1992). Therefore, the study is considered acceptable and the outcome can be reported as valid.
After epidermal induction with 10 % / 25 % glyphosate technical and a challenge treatment with 25 % no skin reactions in any treated or control guinea pigs were observed 48 and 72-hours after the start of the challenge. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Maximisation test.

Assessment and conclusion by RMS:
In this Guinea Pig Maximisation Test, the test substance glyphosate technical (Batch: 206-Jak-25-1, Purity: not reported but assumed to be 98.6%, see details above) had no skin sensitising effect in guinea pigs. The study is

considered acceptable. In the previous evaluation, the study was also considered acceptable (RAR, 2015).

B.6.2.6.21. Study 21

Data point:	CA 5.2.6/021
Report author	[redacted]
Report year	1988
Report title	A Closed-patch Repeated Insult Dermal Sensitization Study in Guinea Pigs (Buehler Method)
Report No	[redacted] 87-218/4470-87
Document No	Not reported
Guidelines followed in study	US EPA Guidelines Section 81-6
Deviations from current test guideline (OECD 406, 1992)	Yes, 10 instead of a minimum of 20 animals used in the treatment group; negative control animals were not treated at all during induction whereas they should have been treated with the vehicle; evaluation of skin reactions 24 and 48 hours instead of 30 and 54 hours after challenge; assessment of sensitivity and reliability of the experimental technique not within six months as recommended in the guideline; DNCB (2,4-dinitro¬chlorobenzene) used as positive control is not one of the preferred substances as recommended in the guideline.
Previous evaluation	According to the applicant, the study was accepted in the previous evaluation (RAR, 2015). From the text in the RAR, however, it cannot be ascertained that the study was indeed accepted. Furthermore, MON 8750 is a preparation/formulation containing 95.2% glyphosate as ammonium salt (corresponding to 86.2% glyphosate acid) and therefore, the study is not addressing the skin sensitising properties of the active substance itself.
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Conclusion GRG: Supportive, Category 2a Conclusion AGG: The study is not considered acceptable for evaluation due to the deviations noted above. It is furthermore not considered relevant to address the skin sensitising properties of the active substance, but only those of a formulation that is not the representative formulation within the current application for renewal.

MON 8750 (Batch: XLH-274; Purity: 86.2 % glyphosate acid) was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler study method. The test-substance concentrations for the main test were selected based on the results of the pre-test. The epidermal induction was performed with undiluted test item moistened with saline under occlusive conditions and was repeated once per week for a total of three applications, each for a duration of six hours. Two weeks after the last induction exposure the animals were challenged by epidermal application of undiluted test material moistened with saline under occlusive dressing for 6 hours.

The study was performed using one control group consisting of five males and five females, and one test group consisting of five animals per sex. None of the animals exhibited a positive skin reaction after the challenge treatment.

DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance in a separately conducted study of the same laboratory. These data demonstrate the susceptibility of guinea pigs to sensitisation.

Based on the results, MON 8750 exhibited no potential to produce dermal sensitisation in guinea pigs.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	MON 8750
Description:	White granular powder
Lot/Batch #:	XLH-274
Purity:	95.2% glyphosate as ammonium salt (corresponding to 86.2% glyphosate acid)
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Distilled water / dinitrochlorobenzene historical control data

3. Test animals:

Species:	Guinea pig
Strain:	Albino Hartley
Source:	[redacted]
Age:	3 – 4 weeks (at receipt); 5 – 6 weeks (at study initiation)
Sex:	Male and female
Weight at dosing:	Male: 366 - 427 g; females: 310 - 370 g
Acclimation period:	16 days (Main study)
Diet/Food:	Agway Prolab Guinea Pig formula provided *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually housed in suspended stainless steel cages
Environmental conditions:	Temperature: Approximately 18 – 24 °C Humidity: 30 – 70 % Air changes: Not reported Photocycle: 12 hours light/dark cycle

B: Study design and methods

In life dates: 1987-09-21 to 1987-11-6

Animal assignment and treatment:

MON 8750 was tested for its sensitising effect on the skin of the guinea pig using a modified Buehler study method. Male and female Dunkin Hartley guinea pigs with body weights ranging from 366 to 427 g for males and 310 to 370 g for females were used.
In the preliminary test, six animals were treated topically with undiluted test material (100 %; moistened with saline) and with concentrations of 50 %, 25 %, and 10 % w/v of the test material in distilled water (4 chambers per animal). Based on results of the pretest, the undiluted material was found to be non-irritating and was, therefore, administered at 100 % concentration for both induction and challenge.

The main study was performed using five animals per sex for the test and the control group, respectively.

Table B.6.2.6.21-1 A Closed-patch Repeated Insult Dermal Sensitisation Study in Guinea Pigs (Buehler Method) [redacted] 1988): Animal assignment to the treatment groups

Treatment group	Number of animals	
Pretest	**Males**	**Females**
Epidermal Pretest	3	3
Main Study	**Males**	**Females**
Test Group	5	5
Irritation Control	5	5

One day prior to the induction phase, the hair was clipped from the back and sides of five male and five female test animals. The next day, the test material was moistened with 0.3 mL saline and 0.3 cm³ of the test substance was applied to the test site. An occlusive dressing was placed over the test site. Approximately six hours after dosing, excessive test material was wiped off. This induction procedure was repeated once per week for a total of three applications. Following each induction, test sites were scored for dermal irritation. Following the induction phase, the test animals were left untreated for a period of 14 days.

On the day prior to challenge hair was clipped from the posterior left side of the test animals and from the untreated guinea pigs. The test substance was administered in the same manner as in the induction phase, but at a second site, on the left side of the midline. After six hours of exposure, the chambers were removed, and the skin wiped free of any excess material.

An additional group of ten animals (five per sex) served as an irritation control group. These animals were not treated during the induction phase and then received the identical challenge dose.

Historical control data from animals exposed to dinitrochlorobenzene was used as positive control.

Body weights were recorded at the start of the main test and on test completion. All animals were observed twice daily for viability and weekly for clinical signs of toxicity.

Skin reaction after treatment was evaluated 24 and 48 hours after each induction and after termination of challenge. Any animal showing erythema at the site of challenge was considered to have shown a positive response. Scores of ± (barely perceptible erythema) were considered equivocal.

The skin reaction to the challenge was scored according to the following criteria:

Score	**Dermal Observations**
0	No reaction
±	Very slight (barely perceptible) erythema, usually non-confluent
1	Slight (well-defined) erythema, usually confluent
2	Moderate erythema
3	Severe erythema, with or without oedema, necrosis or eschar

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed during the study.

B. CLINICAL OBSERVATIONS

No adverse clinical signs of toxicity were observed during the study.

C. BODY WEIGHT

Body weights were considered acceptable.

D. NECROPSY

A necropsy was not performed.

E. SKIN REACTIONS

After induction no dermal irritation was observed.

No skin reactions were observed 24 or 48 h after the challenge treatment with MON 8750 in the control or test group.

Historical control data on the rate of sensitisation after treatment with the positive control DNCB revealed 80 to 100 % sensitised animals, which was considered to sufficiently assure the reliability of this study.

Table B.6.2.6.21-2 A Closed-patch Repeated Insult Dermal Sensitisation Study in Guinea Pigs (Buehler Method) [redacted] 1988): Skin reactions after challenge**

Treatment group	Incidence*	
	24-hours	48 hours
Test group	0/10	0/10
Irritation Control	0/10	0/10

*: Number of animals with findings / number of animals tested
**: Challenge concentration: 25 %

III. CONCLUSIONS

Under conditions of the study, MON 8750 exhibited no potential to produce dermal sensitisation in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant:
The GLP study is in concordance with Guidelines Section 81-6, equivalent to the current OECD 406 (1992). Therefore, the study is considered acceptable and valid. Nevertheless, due to the deviations from the current OECD TG 406 (1992), such as low animal number, the study can be used as supplementary information, only.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, based on the results, glyphosate has no sensitising effect on the skin of the guinea pig in the Buehler Test.

Assessment and conclusion by RMS:
In this Buehler assay, the formulation MON 8750 (Batch: XLH-274; Purity: 95.2% glyphosate as ammonium salt (corresponding to 86.2% glyphosate acid)) had no skin sensitising effect in guinea pigs. Due to deviations from the guideline, however, the study is not considered acceptable for evaluation. It is not certain whether the study was considered acceptable or not in the previous evaluation (RAR, 2015).

B.6.2.6.22. Study 22

Data point:	CA 5.2.6/022
Report author	[redacted]
Report year	1983
Report title	A Dermal Sensitization Study in Guinea Pigs with Glyphosate
Report No	4235-82
Document No	Not reported
Guidelines followed in study	Similar to OECD 406
Deviations from current test guideline (OECD 406, 1992)	10 instead of a minimum of 20 animals used in the test group; age of animals at study initiation not reported; induction treatment on 3 days per week for three weeks instead of once per week for 3 weeks; clinical signs of toxicity not reported; temperature: 21 °C (±3 °C) instead of 20 °C (+ 3 °C).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (not required at the time of study conduct)
Acceptability/Reliability:	Conclusion GRG: Supportive, Category 2a Conclusion AGG: The study is not considered acceptable due to the deviations noted above.

Glyphosate (Batch: NBP 1782610, Purity: 99.7 %) was tested for its sensitising effect on the skin of the guinea pig using a closed patch technique. The test-substance concentrations for the main test were selected based on the results of the pre-test.

During the induction phase, five male and five female animals received 0.2 mL of undiluted glyphosate; an additional five male and five female animals received saline (negative control) and five males and five females received 1-chloro-2,4-dinitrobenzene (positive control). Besides, three animals per sex were used as irritation control for each experimental condition. These animals were not treated during induction, but only at challenge. Applications were made for six hours per day, three days per week for three weeks. Two weeks after the final induction the three male and three female animals exposed to the test material during the induction phase were challenged with 0.2 mL of undiluted glyphosate. The remaining animals served as an irritation control group. Animals in the negative and positive control group also were challenged in a similar manner. Dermal irritation was scored at 24 and 48 hours after each induction application and the challenge application for all animals, including controls. Throughout the study, all animals were observed twice daily for mortality and weekly for clinical signs of toxicity. Body weights were recorded pretest and at termination of the study.

All animals survived until study termination. There were no body weight changes that were considered treatment-related.

No significant irritation was observed after the first five induction exposures to the test material. Beginning on the sixth exposure, very mid irritation was apparent in several animals. Most animals continued to show mild irritation throughout the induction period. These findings were indicative of irritation since no sensitisation occurred during the challenge phase.

All animals challenged with the test material exhibited no dermal response. No dermal responses were observed in negative control animals during the induction and challenge phase.

Positive control animals exhibited slight dermal irritation after the first induction and severe dermal responses beginning after two to three induction exposures. All ten animals showed a positive response after the challenge confirming the sensitivity and reliability of the experimental technique.

Therefore, glyphosate is not considered a skin sensitiser based on the results of a closed patch technique.

I. MATERIALS AND METHODS

A: Materials

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	NBP 1782610
Purity:	99.7% Note: in the previous evaluation (RAR, 2015), the purity of the test item was indicated as 53.8% (IPA salt). The RMS, however, could not retrieve this information regarding purity from the study report. For the current assessment, a purity of 99.7% is assumed.
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Saline / 1-chloro-2,4-dinitrobenzene (DNCB)

3. Test animals:

Species:	Guinea pig
Strain:	Albino Hartley
Source:	[redacted]
Age:	Not reported

Sex:	Male and female	
Weight at dosing:	Male: 420 - 495 g; female: 365 – 451 g	
Acclimation period:	29 days	
Diet/Food:	Charles River Vitamin C-Fortified Guinea Pig Diet, *ad libitum*	
Water:	Automatic watering system, *ad libitum*	
Housing:	Individually housed in suspended stainless steel cages	
Environmental conditions:	Temperature:	18 - 24 °C
	Humidity:	30 - 70 %
	Air changes:	Not reported
	Photocycle:	12 hours light/dark cycle

B: Study design and methods

In life dates: 1982-12-27 to 1983-03-02

Animal assignment and treatment:

Glyphosate was tested for its sensitising effect on the skin of the guinea pig using a closed patch technique, which is similar to the Buehler test method. The test substance concentrations for the main study were selected based on the results of the pre-testing. For the pre-testing, six animals were treated topically with concentrations of 100 %, 50 %, 25 %, and 10 % v/v of the test material in paraffin oil (4 patches per animal). The patches were left in place for six hours, after which they were removed, and the skin was wiped free of any excess material. Based on the results of this study, a 100 % concentration was found to be non-irritating and was therefore, selected for both induction and challenge administration.

The main study was performed in ten test animals and ten negative and positive control animals.

Table 6.2.6-1 A Dermal Sensitisation Study in Guinea Pigs with Glyphosate [redacted] 983): Animal assignment to the treatment groups

Treatment group	Number of Animals	Concentration (%)	
		Induction	Challenge
Group IA – Saline (Negative Control)	10 (5 males, 5 females)	100 %	100 %
Group IB – Saline (Irritation Control)[c]	6 (3 males, 3 females)	---	100 %
Group IIA – DNCB (Positive Control)	10 (5 males, 5 females)	0.5 % [a]	0.3 % [b]
Group IIB - DNCB (Irritation Control) [c]	6 (3 males, 3 females)	---	0.3 % [b]
Group IIIA – Glyphosate	10 (5 males, 5 females)	100 %	100 %
Group IIIB – Glyphosate (Irritation Control) [c]	6 (3 males, 3 females)		100 %

a: Vehicle: 80 % ethanol; b: Vehicle: acetone; c: Irritation control groups were tested at challenge only. The same six animals were used for Groups IB and IIB.

On the day prior to the first application, the hair on the dorsal and lateral surfaces was clipped with an electric clipper. During the induction phase test material (0.2 cm^3) was moistened with 0.2 mL of saline, applied to the skin (right side of the midline) and covered with a gauze square. After administration, an occlusive dressing was used to hold the patch in place. After six hours of exposure, the patch was removed, and the skin was wiped free of excess material. This procedure was repeated three times a week for three weeks, for a total of nine exposures.

Fourteen days after the last exposure, animals were challenged with either the test material or control substance using the same administration procedure used in the induction phase, but on the left side of the midline. After six hours of exposure, patches were removed, and the skin was wiped free of excess material.

Irritation control animals were used to differentiate whether dermal reactions were produced by irritation or sensitisation. Irritation control animals used two groups of three males and three females that were subjected to the same challenge procedures noted above.

Throughout the study, animals were observed twice daily for mortality and weekly for clinical signs of toxicity. Body weights were recorded prior to the start of the study and at study termination.

Dermal scores of 1 or greater was considered clearly indicative of sensitisation. Scores of ± (barely perceptible erythema) were considered equivocal, although a high percentage of scores of ± in treated animals with no dermal response in irritation control animals was considered suggestive of sensitisation.

Dermal responses were scored according to the scale presented in the following:

Score	**Dermal observation**
0	No reaction
±	Very slight (barely perceptible) erythema, usually nonconfluent
1	Slight (well-defined) erythema, usually confluent
2	Moderate erythema
3	Severe erythema, with or without oedema, necrosis or eschar formation

II. RESULTS AND DISCUSSION

A. MORTALITY
All animals survived through the study.

B. CLINICAL OBSERVATIONS
The study report did not provide clinical observations.

C. BODY WEIGHT
All animals, control and treated, gained weight throughout the study. Gains in control and treated animals were considered comparable.

D. NECROPSY
A necropsy was not performed.

E. SKIN REACTIONS
No significant irritation was observed after the first 5 induction exposures to the test material. Beginning on the sixth exposure, very mild irritation was apparent in several animals. Most animals continued to show mild irritation throughout the induction period. These findings were indicative of irritation since no sensitisation occurred during the challenge phase. All animals challenged with the test material exhibited no dermal response.

No dermal responses were observed in negative control animals during the induction and challenge phase.

Positive control animals exhibited slight dermal irritation after the first induction and severe dermal responses beginning after two to three induction exposures. All 10 animals showed a positive response after the challenge confirming sensitisation.

Table 6.2.6-2 A Dermal Sensitisation Study in Guinea Pigs with Glyphosate [redacted] 1983): Summary of skin reactions after challenge

Treatment group	Interval (hr)	Dermal scores					Total number of animals
		0	±	1	2	3	
IA – Saline (Negative Control)	24	10	0	0	0	0	10
	48	10	0	0	0	0	10
IB – Saline (Irritation Control)	24	6	0	0	0	0	6
	48	6	0	0	0	0	6
IIA – DNCB (Positive Control)	24	0	0	2	8	0	10
	48	0	0	7	3	0	10
IIB - DNCB (Irritation Control)	24	4	2	0	0	0	6
	48	6	0	0	0	0	6
IIIA – Glyphosate	24	10	0	0	0	0	10
	48	10	0	0	0	0	10
IIIB – Glyphosate (Irritation Control)	24	6	0	0	0	0	6
	48	6	0	0	0	0	6

III. CONCLUSIONS

Under conditions of this study, glyphosate revealed no dermal sensitisation in guinea pigs.

Assessment and conclusion

Assessment and conclusion by applicant
The study is similar to the current OECD 406 (1992). The study is considered acceptable and valid. Nevertheless, due to the deviations, such as reduced test animal number, different test method, non-GLP, the study can be used as supplementary information, only.
After challenge treatment with glyphosate, no skin reactions in guinea pigs were observed after 24 and 48 hours. Therefore, glyphosate is not considered a skin sensitiser based on the results of a closed patch technique.

Assessment and conclusion by RMS:
In this Buehler assay with nine inductions, the test item glyphosate (Batch: NBP 1782610, Purity: 99.7%) had no skin sensitising effect in guinea pigs. Due to deviations from the guideline, however, the study is not considered acceptable for evaluation. It is not certain whether the study was considered acceptable or not in the previous evaluation (RAR, 2015).

B.6.2.6.23. Study 23

Data point:	CA 5.2.6/023
Report author	Lindberg T. *et al.*
Report year	2020
Report title	An integrated transcriptomic- and proteomic-based approach to evaluate the human skin sensitization potential of glyphosate and its commercial agrochemical formulations
Document No	Journal of proteomics, (2020) Vol. 217, pp. 103647
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	No
Acceptability/Reliability:	**Conclusion GRG:** Yes / Reliable with restrictions **Conclusion AGG:** The study is considered to be reliable with restrictions.

Full summary of the study according to OECD format

The authors investigated the skin sensitization hazard of glyphosate, the surfactant polyethylated tallow amine (POEA) and two commercial glyphosate-containing formulations using different omics-technologies based on a human dendritic cell (DC)-like cell line. First, the GARD™skin assay, investigating changes in the expression of 200 transcripts upon cell exposure to xenobiotics, was used for skin sensitization prediction. POEA and the formulations were classified as skin sensitizers while glyphosate alone was classified as a non-sensitizer. Interestingly, the mixture of POEA together with glyphosate displayed a similar sensitizing prediction as POEA alone, indicating that glyphosate likely does not increase the sensitizing capacity when associated with POEA. Moreover, mass spectrometry analysis identified differentially regulated protein groups and predicted molecular pathways based on a proteomic approach in response to cell exposures with glyphosate, POEA and the glyphosate- containing formulations. Based on the protein expression data, predicted pathways were linked to immunologically relevant events and regulated proteins further to cholesterol biosynthesis and homeostasis as well as to autophagy, identifying novel aspects of DC responses after exposure to xenobiotics.

The RMS notes the following: next to glyphosate, the authors also investigated skin sensitising properties of POEA (polyethoxylated tallow amine) and glyphosate formulations containing POEA. Since POEA, as well as formulations containing this adjuvant, are no longer allowed in the EU, the corresponding results are not shown in the study summary presented below as they are not relevant for risk assessment.

Materials and methods

Chemical and reagents
Dimethyl sulfoxide (DMSO), p-phenylene-diamine (PPD), HyClone™ minimum essential medium alpha modification with L-glutamine, ribo- and deoxyribonucleosides (MEM-α) and TRizol® were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) was purchased from PeproTech (Rocky Hill, NJ, USA). Propidium iodide (PI), FITC-conjugated anti-human [CD86 (FUN-1), HLA-DR (L243), CD34 (581) and isotype control anti- IgG1 (MOPC-21)] and PE-conjugated anti-human [CD54 (HA58), CD80 (L307) and isotype control anti-IgG1 (MOPC-21)] antibodies were acquired from BD Biosciences (San Jose, CA, USA). FITC-conjugated anti-human CD1a (NA1/34) and PE-conjugated anti-human CD14 (TÜK4) antibodies were purchased from Dako (Santa Clara, CA, USA). Direct-zol™ RNA MiniPrep column purification kit and trypsin were acquired from Zymo Research (Irvine, CA, USA) and Promega Biotech AB (Madison, WI, USA), respectively. Hybridization buffer, reporter co-deset, capture probeset and nCounter® cartridges were purchased from NanoString® Technologies (Seattle, WA, USA). Protease inhibitor tables EDTA-free and Silica C18 UltraMicro spin columns were acquired from Roche Diagnostics GmbH (Mannheim, Germany) and The Nest group (Southborough, MA, USA), respectively. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture
MUTZ-3-derived cells (DSMZ, Braunschweig, Germany) were cultured in MEM-α supplemented with 20% (v/v) fetal bovine serum (FBS) and rhGM-CSF (40 ng/mL), and maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C. Cell viability was analyzed using LUNA™ automated cell counter (Logos Biosystems, Annandale, VA, USA), according to manufacturer's instructions. A cell viability value > 90% was considered satisfactory to carry out the experiments, which were performed using three independent batches of cells. Moreover, quality control analysis was performed for each cell batch using BD FACSCanto II flow cytometer (Biosciences, San Jose, CA, USA). For that, the following antibodies were used: FITC-conjugated anti-human CD1a, CD34, CD86 or HLA-DR, and PE-conjugated anti-human CD14, CD54 or CD80; FITC- or PE-anti-IgG1 were used as isotype controls, while staining with PI (1 μg/mL) was performed for cell viability analysis.

Cell exposure with the test materials
Cell exposures and selection of input non-cytotoxic concentration of each test material (e.g. test material concentration inducing 90% relative viability, when compared to unexposed cells, namely RV90 value) were performed as follows: test materials were diluted in appropriate vehicles (Table 1) and the cells (2×10^5 cells/mL) were exposed to different concentrations for 24 h. After that, flow cytometer analysis was conducted to evaluate the cell viability using PI staining and then to obtain the RV90 values. In the case of non-cytotoxic materials, an input concentration of 500 μM was used. After that, cells were exposed to each test material at input concentration (Table 1) to carry out the flow cytometric analysis of CD86 expression and transcriptional analysis. Cell exposures were performed in triplicate cell batch reactions, i.e. a new cell batch was used each exposure round, resulting in a total of 18 samples. Total RNA was collected by lysing 2×10^5 cells/exposure in TRIzol® and stored at −20 °C until RNA purification and further processing for transcriptional analysis and GARD™ skin predictions.

Table 6.2.6.23-1: Test materials used for cell exposures.

Material	Abbreviation	CAS no.	Vehicle	Classification	Input concentration
Double-distilled water	H_2O	na	na	Unexposed	0.1% (v/v)
Dimethyl sulfoxide	DMSO	67-68-5	na	Vehicle control	0.1% (v/v)
p-Phenylenediamine	PPD	106-50-3	DMSO	Positive control	75 μM
Glyphosate	GLY	1071-83-6	H_2O	na	500 μM

na: not applicable

GARD™skin assay
The *in vitro* assay GARD™skin (SenzaGen AB, Lund, Sweden) was used to predict the ability of chemical substances to induce skin sensitization. Total RNA was isolated from the cells lysed in TRIzol® reagent using the Direct-zol™ RNA MiniPrep column purification kit (Zymo Research), according to manufacturer'

instructions. For each sample, concentration and RNA integrity was determined with an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA). The following gene expression analysis was performed on NanoString® GEN2 nCounter analysis system (NanoString® Technologies) using the GARD™skin assay. In brief, 5 µL RNA sample were mixed with 8 µL master mix, comprising hybridization buffer and reporter CodeSet, and 2 µL Capture ProbeSet followed by 24 h hybridization at 65 °C using a 3Prime thermal cycler (Bibby Scientific, Staffordshire, UK). Samples were then processed and transferred to a 12-well nCounter® cartridge in the GEN2 Prep station 5s set at high sensitivity followed by data collection on the Digital Analyzer 5s with an imaging resolution of 555 fields of view. The Reporter Code Count files (RCC) files were then downloaded from the Digital Analyzer and processed using SenzaGen software version 1.0 (SenzaGen AB), which conducts both NanoString® quality control and data normalization. A support vector machine (SVM) algorithm was used to assign a so-called decision value (DV) to each test material replicate. A test material is classified as skin sensitizer when the SVM median output value is > 0 of the three independent replicates, while a median DV < 0 classifies the material as a non-sensitizer.

Proteomic analysis

Protein extractions and concentration determination

For proteomic analysis, cells were also exposed to each test material at input concentration (Table 1). After 24 h, cells were harvested and processed for mass spectrometry (MS) analysis by pelleting the chemically exposed cells in a maximum recovery Eppendorf tube followed by washing with phosphate buffered saline (PBS) before flash freezing of the pellet in liquid nitrogen. Cell pellets were stored at −80 °C before further MS processing. Each cell batch of chemical exposure was performed in duplicates of 2 × 106 cells/exposure, and thereafter pooled to ensure a sufficient protein amount for MS analysis. In addition, triplicates of unexposed (water) samples were run for MS analysis. Following cellular exposure proteins were extracted from each sample by lysing the cells in 150 µL lysis buffer (8 M urea, 39 mM Tris, 5 mM MgAc, 4% CHAPS, protease inhibitor tablet) through three freeze/thaw cycles and separating supernatant and cell debris by centrifugation at 16000 ×g for 30 min. Protein extracts were stored in −80 °C until further processing. Protein concentration determination was performed using the Total Protein Micro-Lowry kit (Sigma-Aldrich) according to manufacturer's instructions. The concentration was determined by measuring the absorbance at 650 nm and 50 µg per sample were used for further processing.

In gel trypsin digestion and mass spectrometry

Proteins were separated on an SDS-PAGE gel and digested into peptides using trypsin. Trypsinated peptides were then de-salted using UltraMicro spin columns (Silica C18, SUM SS18 V, Nest group) as described in Chawade et al.. Peptides were thereafter resuspended in 0.1% formic acid (FA) and loaded into an EASY-nano liquid chromatograpjy (LC) system 1200 (Thermo Fisher Scientific). Peptides were inected directly into the analytical column, a 15 cm long fused silica capillary (75 µm × 16 cm Pico Tip Emitter, New Objective, Woburn, MA, USA) packed in-house with C18 material ReproSil-Pur 1.9 µm (Dr Maisch GmbH, Germany). Peptides were separated using an 80 min gradient from 5% to 90% solvent B (80% acetonitrile, 0.1% FA, v/v) at a constant flow rate of 250 nL/min. The nLC system was coupled with a Q-Exactive™ HF-X (Hybrid Quadrupole-Orbitrap™) (Thermo Fisher Scientific) operated in a positive mode for data-dependent acquisition (DDA). The Orbitrap acquired the full MS scan with an automatic gain control (AGC) target value of 3 × 106 ions and a maximum fill time of 50 ms. The 20 most abundant peptide ions were selected from the MS for higher energy collision-induced dissociation (HCD) fragmentation (collision energy: 40 V). The instrument was scanning at a target MS1 resolution of 120,000 between 375 and 1500 m/z window with 15,000 MS/MS resolution for a target of 1 × 105 and a maximum injection time of 20 ms using an isolation window of 1.2 m/z.

Results

GARD™skin ASSAY

Following 24 h of exposure with pure glyphosate, cytotoxicity analysis was performed using PI staining to define the input concentration for each test material (Table 6.2.6.23-1). Cells were then exposed to the test materials at input concentrations for 24 h and the maturity state of the cells was assessed by measuring levels of cell surface expression of the co-stimulatory marker CD86. Only the positive control PPD induced a significant upregulation of CD86 expression ($p < 0.05$) in comparison to unexposed control. Cell exposure with pure glyphosate resulted in a modest downregulation compared to control ($p < 0.05$). No significant difference between DMSO and unexposed controls was observed.

Furthermore, skin sensitization hazard predictions were performed with the GARD™skin assay. Transcriptomic analysis of the 200 bio-markers from the GARD™skin prediction signature was carried out using the

NanoString® nCounter™ System. The skin sensitizing hazard was predicted using an SVM algorithm where each test material replicate was given a DV. As expected, PPD and DMSO were correctly classified as skin sensitizer and non-sensitizer, respectively. Also, glyphosate was classified as a non-sensitizer with a mean SVM DV of −1.45.

Profiling of agrochemical-induced changes in the cellular proteome
Initially, peptide data, generated from the trypsin-digested cells exposed with test materials for 24 h were normalized and assembled to protein groups. PCA evaluation was used to identify patterns, i.e. which samples exhibit a similar protein expression profile. As a first step, unsupervised clustering of all protein groups was performed. A clear separation between sensitizers (PPD) and non-sensitizers (unexposed, DMSO, glyphosate) is observed. Additionally, by applying a multi-group comparison (FDR = 0.05) based on all the treatments, i.e. clustering of the individual treatments most alike based on their protein expression, a similar pattern was observed as when unsupervised clustering was applied.

Conclusions

The objective of this study was to perform *in vitro* sensitization testing with a parallel MS-based proteomic approach to investigate the underlying cellular mechanisms induced. The experimental setup of the *in vitro* assay GARD™skin was used to stimulate cells of a human DC-like cell line with different materials, including glyphosate. As a first step, the DC maturation marker CD86 was investigated. While the positive control PPD led to a significant upregulation of CD86, glyphosate induced a significant downregulation of CD86. Furthermore, the skin sensitizing capacity of the test materials was determined. Glyphosate was predicted as a non-sensitizer.
Furthermore, the transcriptomic data were complemented with a proteomic analysis. The assembled protein groups, deduced from the peptides obtained from the MS analysis resulted in a clear separation between sensitizers (PPD) and non-sensitizers (unexposed, DMSO, glyphosate).

Assessment and conclusion by applicant:
Investigation of molecular mechanisms in the skin sensitization process specifically focusing on DC activation using an integrated transcriptomic- and proteomic approach.
First, Mutz-3-derived cells were exposed to PPD, DMSO, unexposed sample and glyphosate. No cytotoxicity was observed for glyphosate and glyphosate was classified as non-sensitising.
Second, PPD, DMSO, unexposed sample and glyphosate were assembled to protein groups. A clear separation between sensitizers (PPD) and non-sensitizers (unexposed, DMSO, glyphosate) was observed. Data on glyphosate are consistent with other available validated assay results.

The article is classified as reliable with restrictions for the following reason: This is a non-validated test system. The purity and origin of glyphosate is unclear. only 1 dose tested (no dose relationship can be evaluated), no HCD were available in order to compare with the equivalent concurrent controls and test groups results.

Reliability Criteria as defined by the applicant: *In Vitro* Toxicology Studies		
Publication: Lindberg T. *et al.* 2020	**Criteria met? Y/N/?**	**Comments**
Guideline-specific		
Study in accordance to valid internationally accepted testing guidelines	N	Not applicable because there is no OECD guideline for this type of test
Study performed according to GLP	N	
Study completely described and conducted following scientifically acceptable standards	Y	
Test substance		
Test material (Glyphosate) is sufficiently documented and reported (i.e. purity, source, content, storage conditions)	N	The only information is that glyphosate's input concentration was 500 µM. The purity, origin

		etc. is unclear.
Only glyphosate acid or one of its salts is the tested substance	N	Glyphosate alone and EU non-representative formulations were tested (summary above is provided only for glyphosate)
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	n.a.	Not applicable as no metabolic activation system used
Test concentrations in physiologically acceptable range (< 1 mM)	Y	
Cytotoxicity tests reported	Y	
Positive and negative controls	Y	
Complete reporting of effects observed	Y	
Statistical methods described	Y	
Historical negative and positive control data reported	N	
Dose-effect relationship reported	N	Not applicable as only 1 concentration was used
Overall assessment		
Reliable without restrictions	N	
Reliable with restrictions	Y	This is a non-validated test system. The purity and origin of glyphosate is unclear. Only 1 dose tested (no dose relationship can be evaluated). No HCD were available in order to compare with the equivalent concurrent controls and test groups results.
Not reliable	N	

Assessment and conclusion by RMS:
The RMS agrees with the summary and assessment as presented by the applicant. Furthermore, it is agreed to considered the study as reliable with restrictions due to the limitations reported above.

B.6.2.7. Phototoxicity

According to Regulation (EU) No 283/2013 a phototoxicity test is required if the substance absorbs in the range of 290-700 nm and is liable to reach the eyes or light-exposed areas of skin. No UV/VIS maximum was observed at wavelengths of >250 nm. And the ultraviolet/visible molar extinction/absorption coefficient of glyphosate is smaller than 10 L/(mol*cm) at wavelenghts of ≥ 290 nm (refer to Volume 3 CA B.2 at section CA 2.4/002). Therefore, no phototoxicity study was performed with glyphosate.

B.6.3. SHORT-TERM TOXICITY

Refer to separate RAR B.6.3.

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. NEUROTOXICITY

Refer to separate RAR B.6.7-6.10

B.6.8. OTHER TOXICOLOGICAL STUDIES

Refer to separate RAR B.6.7-6.10

B.6.9. MEDICAL DATA AND INFORMATION

Refer to separate RAR B.6.7-6.10

B.6.10. REFERENCES RELIED ON

Refer to separate RAR B.6.7-6.10